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AN INTRACELLULAR MICROELECTRODE STUDY OF THE MECHANISM OF ORIGIN OF RHYTHMIC MUSCLE FIBER ACTIVITY

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Investigations of the electrical activity of the nervous system show that many excitable elements (cells, axons, receptors) are able to generate rhythmic discharges in response to single stimuli. A suitable model for the study of rhythmic activity is the spontaneous electrical activity which develops in striated muscle fibers when the muscle is placed in Ringer solution with a reduced calcium concentrate.

METHODS

Experiments were carried out on isolated nerve-muscle preparations of the sartorius muscle of the frog Rana pipiens. The muscle was placed in a chamber containing modified Ringer solution in which the concentration of calcium chloride was reduced to 20 - 50 per cent of normal. Muscle potentials were recorded with glass intracellular microelectrodes by the standard method [2, 8]. The motor nerve was stimulated with rectangular impulses from an electronic generator.

RESULTS

Muscles placed in the solution with a reduced concentration of calcium ions showed spontaneous rhythmic activity. When recorded with intracellular microelectrodes, the activity of single fibers took two main forms: spike potentials, and slow waves of negativity with spike potentials superimposed (Fig. 1a and c). In some instances, slow waves appeared that were not accompanied by spikes. As a rule, each spike was preceded by an independent slow, local negative potential change—the prepotential [3]—regardless of whether the spike developed at the peak of a slow wave of negativity or without any such slow wave. In some cases prepotentials were absent, which may be explained by the fact that the spontaneous spikes being recorded had developed some distance away from the point where the microelectrode entered the cell, so that the local variations in potential accompanying the spikes were not detected. The propagated spike was able to generate new waves of activity, with clearly distinguishable prepotentials (Fig. 1c) near the site of insertion of the electrode into the cell. The rhythmic activity recorded from different fibers was marked by great variability, as regards both the frequency and the duration of the waves. Some fibers were capable of rhythmic activity over a period of many seconds, while in others rhythmic activity stopped after a few discharges had appeared. The cause of this dying out of rhythmic activity is not clear. It might be connected either with cessation of the activity of the stimulating focus that gives rise to the observed rhythm, or with elevation of the fiber's threshold of excitability to the stimulating action of the focus of excitation [4]. Data are also available to indicate that in the absence of sufficient ionized calcium, there is a gradual inactivation of the process of migration of sodium ions into the cell and expulsion of sodium ions out of the cell during the recovery period by the "sodium pump" [7]. For this reason, it is of great interest to determine the fiber's capacity for activity immediately after its rhythmic activity stops.

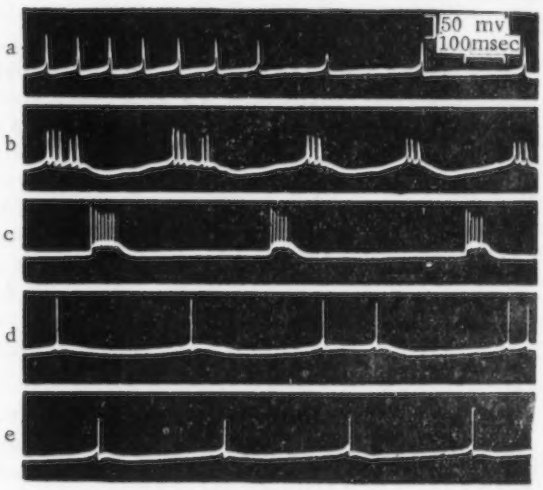

Fig. 1. Rhythmic activity of a muscle fiber. a, b, c) Spontaneous activity; d) rhythmic activity evoked by application of Ditilin (succinylcholine iodide); e) rhythmic activity evoked by nerve impulse.

For this purpose we investigated the effects of certain substances that are able to excite muscle fibers. It was found that such substances as decamethonium, Ditilin (succinylcholine), tetramethylammonium, veratrine, and caffeine, when added to calcium-deficient Ringer solution, almost always cause rhythmic activity to appear in cells that were inactive before the application of these preparations. The activity produced by application of depolarizing substances was indistinguishable in character from spontaneous activity (Fig. 1d). When calcium ions were added to the solution bathing the muscle, depolarizing substances produced rhythmic activity in only a small number of fibers. Curarization (tubocurarine chloride in a concentration of 5×10^{-6}) almost always prevented the appearance of activity in response to the application of tetramethylammonium, Ditilin, or decamethonium, and had little effect on the action of veratrine and caffeine. It follows that the development of rhythmic activity under the influence of substances in the first group is primarily related to their selective depolarizing action on the endplate region, but the effect of veratrine and caffeine is explained by depolarization of the remainder of the muscle fiber surface. Thus, rhythmic activity may arise as the result of prolonged depolarization of different regions of the cell: the endplate and the electrically excitable membrane.

An interesting feature of the action of depolarizing substances was their capacity to evoke rhythmic activity in fibers in which spontaneous activity had stopped before the preparation was applied. In this case, the potential variations which developed in response to the depolarizing substances sometimes corresponded exactly to the original potential variations, and sometimes differed markedly from them both in frequency and in the form and amplitude of the recorded potentials. This indicates that the cessation of rhythmic muscle fiber activity is not the result of inactivation of processes of active transport of sodium ions. It is probable that activity stops as a result of accommodation of the fiber to the stimulating action of the focus of excitation [1]. Moreover, we may suppose that the secondary rhythmic activity produced by the depolarizing substance, when it is identical to the initial spontaneous activity, is a consequence of depolarization of exactly the same region of the cell in which the rhythmic activity had arisen spontaneously. When changes occur in the form and frequency of the potentials, we may suppose that the preparation has a depolarizing effect on some other region of the cell, which now becomes the "pacemaker."

In order to test the assumption that spontaneous rhythmic activity arises as a result of activation of individual regions of the cell, from which the excitation process then passes to the rest of the fiber in the form of local and propagated potentials, experiments were performed with electrical stimulation of the motor nerve of the muscle being investigated.

It is well known that when ionized calcium is absent from the solution bathing the muscle, neuromuscular transmission is blocked [6]. Even so, under conditions where the calcium concentration in the Ringer solution was reduced to 20–50 per cent of normal, we were still able to record endplate potentials as well as spike discharges of the muscle fibers. The muscle retained its ability to display spontaneous rhythmic activity.

Stimulation of the motor nerve was often accompanied by development of prolonged rhythmic activity in "quiescent" fibers, which remained even after stimulation was discontinued. This activity completely corresponded in character to spontaneous rhythmic activity (Fig. 1e). Stimulation of the nerve when spontaneous activity was already present caused the spontaneous rhythm to be eliminated, and to be replaced by a rhythm synchronized with that of stimulation (Fig. 2a and a_1). In other instances, stimulation was followed by cessation of previous activity. Thus, a spontaneously active muscle fiber has the distinct capacity to react to excitation propagated from the endplate region. In some cases the muscle fiber does not respond immediately to nerve stimulation, but assumes the rhythm of stimulation gradually, at first giving potentials that are irregular and complex in form (e.g., the paired discharges in Fig. 2b and b_1), which then change, as stimulation continues, into spikes of the usual form, strictly synchronized with the stimulating rhythm.

Finally, when rhythmic activity has developed in the fiber, it may proceed quite independently of the rhythm of nerve stimulation (Fig. 2c and c_1). But usually even in these cases uninterrupted stimulation ultimately leads to synchronization of muscle potentials with the rhythm of stimulation.

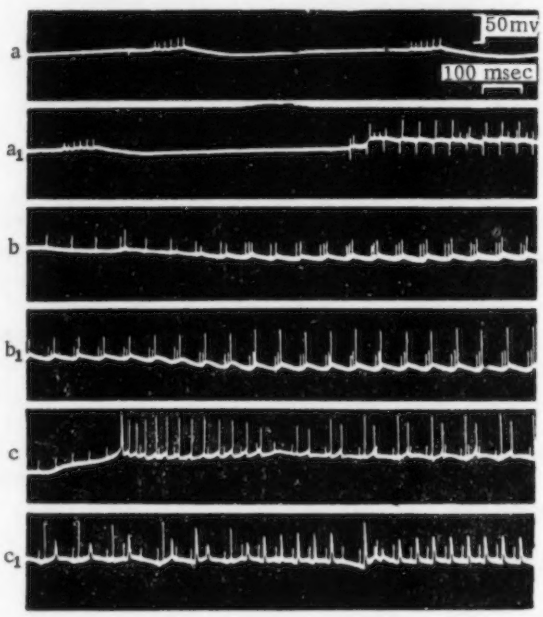

Fig. 2. The effect of stimulation of the motor nerve on rhythmic activity of the muscle fiber.
a, a_1) Effect observed with spontaneously active muscle fibers; b, b_1 and c, c_1) effect observed in absence of automatic activity.

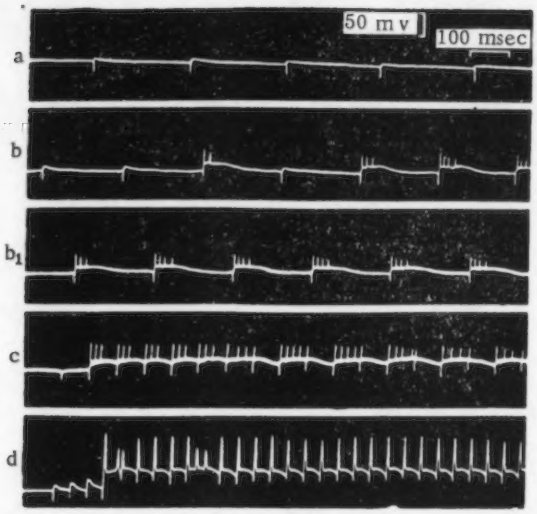

Fig. 3. Effect of frequency of motor nerve stimulation on character of rhythmic activity of muscle fiber. a,b,c,d) Rhythmic activity of fiber with increasing frequency of stimulation. Microelectrode in endplate region: b,b_1) continuous recording during repeated stimulation.

We were interested in the dependence of rhythmic muscle responses on the frequency of electrical stimulation of the motor nerve. It was found that there exists a definite optimum range of frequencies (10–50 c/s), at which rhythmic responses synchronous with stimulation developed in a muscle fiber.

The significance of the stimulation frequency is illustrated in Fig. 3, which shows recordings of muscle potentials with an intracellular microelectrode inserted in the region of the endplate. The magnitudes of the endplate potentials were greatly reduced in response to a reduced calcium ion concentration. At a slow stimulus frequency only local potentials develop, which do not give rise to spikes. With increase in frequency of stimulation, rhythmic discharges occur in response to some stimuli, and become more and more stable as tetanic stimulation continues. As the stimulus frequency continues to increase, this process is accelerated, and at the beginning of the stimulus the muscle begins to show rhythmic responses after each nerve impulse. As the rate of stimulation increases still further, assimilation of the rhythm develops rapidly but the rhythmic character of each response is lost, and the muscle develops a single spike in response to almost every nerve impulse.

This relationship between the rhythmic responses of the muscle and the frequency of nerve firing can be explained by the fact that at low rates of stimulation the endplate potentials are too small to excite the rest of the muscle fiber surface. With increase of stimulus frequency, the endplate potentials become larger (postactivation potentiation), and the level is reached which is necessary for excitation of the neighboring portion of the membrane, in which rhythmic responses are generated. As the size of the endplate potentials increase further, the muscle fiber generates only single responses, since a large depolarization depresses the capacity of the fiber to give rhythmic spike potentials, although in calcium deficiency a successive increase in the endplate potentials certainly occurs in the course of tetanic stimulation [6] and is seen in the illustration presented (see Fig. 3).

Nevertheless, the possibility is not excluded that assimilation of the stimulus rhythm by the muscle fiber, particularly in the presence of spontaneous muscle fiber activity, may also depend on other causes. By analogy with data obtained on smooth muscle [5], which behaves very much like striated muscle in a calcium-deficient solution, we can assume that assimilation of the stimulus rhythm by a fiber that is capable of spontaneous activity is related to its capacity to generate slow waves. Actually, group discharges developed, as a rule, both to spontaneous slow negative potential variations and to slow waves produced by a nerve impulse. We have not made direct determinations of fiber refractoriness, but the fact that a subsequent discharge may develop in the fiber, even when the effective stimulus coincides with the falling phase of the previous spike or comes immediately after the end of this phase, shows that the refractory period is very brief and does not exceed 1–3 msec. Therefore, the refractory period does not determine the muscle fiber's capacity to assimilate the stimulus rhythm and to respond to a single impulse with a rhythmic discharge.

Thus, the data we have presented show that rhythmic muscle fiber potential variations in a calcium-deficient medium may arise as a result of local depolarization of the fiber endplate under the influence of various pharmacological substances or of a nerve impulse, and also as a result of a depolarizing influence on the rest of the muscle fiber surface. When rhythmic discharges are produced by a nerve impulse, the frequency of nerve firing is of great importance.

SUMMARY

Experiments were performed on an isolated nerve-muscle preparation of frog sartorius muscle kept in a solution with reduced calcium ion concentration. The appearance of rhythmic activity of the muscle fibers may be caused by depolarization of either the endplate area or other portions of the membrane. Depolarization resulting in rhythmic activity may be caused by the application of some pharmacological substances (succinylcholine, decamethonium, tetramethylammonium, caffeine, veratrine) or by a nerve impulse. The involvement of the muscle fiber in rhythmic activity is especially effective when the motor nerve is stimulated at a frequency of 10 to 50 stimuli per second.

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REFLEXES EVOKED BY RHYTHMIC STIMULATION OF MUSCLE NERVES

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As we all know, when skeletal muscles are stretched, either brief reflex responses (tendon reflexes) or prolonged, steady contractions result. The phasic stretch reflexes have been quite thoroughly studied [3, 4, 9]; but efforts to make use of the data obtained in this way to characterize static strength reflexes, which are of immeasurably greater biological importance, have run up against serious difficulties [5, 8, 13]. In the papers referred to, it was established that static reflexes evoked by rhythmic stimulation of the afferent fibers of muscle nerves can not be regarded as combinations of phasic responses. In contrast to the phasic reflexes, static stretch reflexes require for their development the presence of an initial level of motoneuron activity, as well as a relatively high stimulus frequency—conditions that favor summation of successive afferent impulses. In view of these facts, further study of static proprioceptive reflexes is needed. In particular, there is considerable interest in obtaining an answer to the question raised by N. E. Vvedenskii, regarding the effect of strength and frequency of stimulation on the course of reflex reactions.

In the present paper, we will set forth data on the way reflex excitation and inhibition of extensor muscles depend on the strength of rhythmic stimulation of the central ends of muscle nerves, and on the relation of these phenomena to excitation of particular groups of nerve fibers.

METHODS

The experiments were carried out on 40 decerebrate cats, with marked decerebrate rigidity. In both hind limbs the muscles that move the knee joint were denervated, with the exception of the quadriceps muscles. In the right limb, the nerve to the deep and medial heads (vasti cruralis et medialis) of the quadriceps femoris, or the nerve to the deep head alone, was exposed and sectioned. Immersion electrodes (Ag—AgCl, interelectrode distance 3 mm) were applied to the central end of this nerve (the words "central end" will be omitted hereafter). In some experiments an indifferent anode was used. The nerves were stimulated with electrical impulses of rectangular form (duration 0.07 msec, or sometimes 1 msec), from a stimulator with a stimulus isolation unit. Starting and stopping of the kymograph, and application of stimuli, were automatic. Stimuli were applied for two-second periods, at one-minute intervals. The stimulus intensity is indicated throughout as percent of threshold intensity.

The animal was placed in the supine position, and the femoral bones were fastened in the vertical position. Movements of the tibia (changes in the angle between the femur and the tibia), reflecting changes in the tone of the quadriceps, were recorded by means of isotonic myographs.

RESULTS

Rhythmic stimulation of the muscular branches of the femoral nerve were capable of producing both reflex contraction and deep inhibition of the tone of those heads of the ipsilateral quadriceps that were still innervated (Fig. 1). Contractions of significant magnitude developed only in response to stimulation at a rather high frequency (above 50 per second); accordingly, a stimulus frequency of 100 per second was employed. These contractions were like an enhancement of tone in character—i.e., the curves rose smoothly to a maximum, and remained at this level, in the form of a plateau, as long as stimulation continued (more than 10 minutes: Fig. 1a). Rapid, short contractions preceding the main response were observed only now and then, in response to relatively intense stimulation. But these contractions must be regarded as a response to the first afferent volley, since they disappeared or diminished when the stimulus frequency was reduced. In most cases, stimulation produced reflex contraction of the contralateral quadriceps (Fig. 1).

When impulses were applied to the nerve to the deep and medial heads of the quadriceps while the animal was in a condition of marked decerebrate rigidity, distinct reflex reactions were always observed. The maximum contraction seen upon stimulating this nerve for two seconds corresponded to extension of the knee joint of from three to 25 degrees, in the various experiments. For the smaller nerve to the deep head, the contractions were smaller (1—15 degrees) and occurred in only about half of the experiments. When both nerves were stimulated for longer periods, much stronger contractions were sometimes seen (extension of 30—50 degrees).

The intensity of the reflex responses was found to depend on the intensity of the stimulus applied to the muscle nerves. An increase in stimulus amplitude above threshold was accompanied by an increase in the height of contraction to a certain maximum level (Fig. 1b). In some experiments the stimulus intensity that produced the strongest contraction when applied to the nerve to the deep and

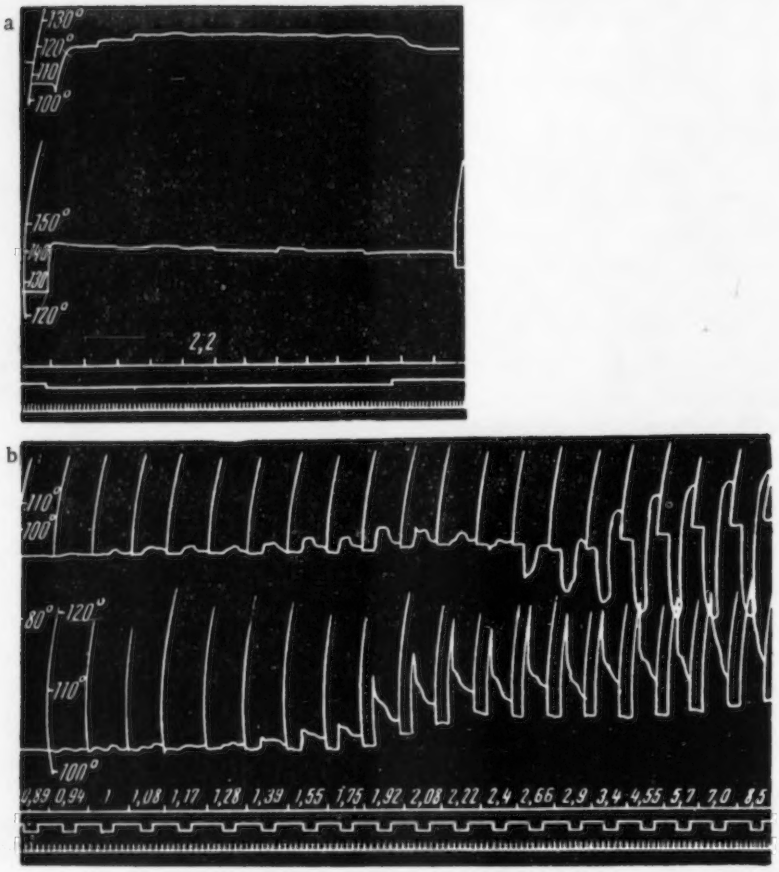

Fig. 1. Quadriceps reflexes in response to stimulation of the central ends of muscle nerves. Interpretation of records (top to bottom): movement of right (ipsilateral) tibia; movement of left tibia (upward movement corresponds to extension); mark indicating when kymograph was stopped (for one minute); stimulation of nerve to deep and medial heads of quadriceps; time marker (1 second). The vertical scale indicates the position of the limb, in degrees of angle between femur and tibia. a) prolonged stimulation (more than 10 minutes); b) stimulation for two seconds. Figures indicate intensity of stimulation: 0.89; 0.94; 1.0; 1.08; 1.17; 1.28; 1.39; 1.55; 1.75; 1.92; 2.08; 2.22; 2.4; 2.66; 2.9; 3.4; 4.55; 5.7; 7.0; 8.5 (with respect to threshold).

medial heads was between 1.4 and 2.4 times the threshold intensity (42 out of 50 determinations on 17 animals). Maximal contractions were particularly often observed at intensities of 1.8–2.2 times threshold intensity (19 determinations).

A further increase in the stimulus intensity usually resulted in lessening of the height of contraction, and then, in replacement of contraction by relaxation (see Fig. 1b). In these cases the end of stimulation was often accompanied by a return to the initial state. This sort of relationship between the height of response and the stimulus intensity was recorded in 22 out of 29 instances. The minimum stimulus intensities at which relaxation of the muscle below the initial level occured were 2–3.2 times the threshold intensity in most cases. In response to more intense stimuli, inhibition became more profound, and reached its greatest depth at stimulus intensities of 4–8 times threshold, or more.

In some animals reflex contractions was more pronounced, in others reflex inhibition, and in a third group both the positive and negative reactions were marked. This is indicated by the wide scatter of stimulus intensities marking the transition from contractions to relaxations. In five cases, there was no clear-cut display of inhibition,

even when the stimulus intensity became very great. In some experiments, on the other hand, the contractions were very weak, but profound inhibition of the quadriceps developed readily.

In order to establish the connection between the character of reflex responses and stimulation of particular groups of afferent nerve fibers, it was necessary to compare thresholds for reflexes with those of afferent fibers of muscle nerves.

For observation of dorsal root action potentials, the animals were placed in a special stand, in the prone position. After laminectomy of the lumbar vertebrae, repeated determinations of the thresholds of reflex excitability were made. Then the dura mater was opened and a bundle of fibers from the fifth or sixth lumbar dorsal root was lifted with a ligature and placed across the recording electrodes. The roots were covered with liquid petrolatum. Then the thresholds of excitability of these afferent fibers were determined several times. Action potentials were recorded with the sweep frequency of the cathode-ray oscillograph synchronized with the rhythm of stimulation at 100 per second. Distances between the stimulating cathode and the recording electrode were measured on isolated nerves while they were tightly stretched.

It was found that when decerebrate rigidity was sufficiently marked, the thresholds of reflex responses were only slightly greater than those of the most excitable afferent fibers. In four experiments, the following mean values were obtained for the differences between these thresholds (in percents): 5, 1.5, 0, and 2. These differences are within the error of the determination of stimulus intensities corresponding to the different forms of reflex responses. We can therefore suppose that in most experiments the thresholds of reflex excitability rather closely reflected the thresholds of the afferent fibers. We might mention that for single stimulations of muscle nerves the differences between these thresholds are far greater [7].

The fact that the compound dorsal root action potential clearly displays several peaks when the muscle nerve is stimulated (Fig. 2) is in agreement with published data [7]. The relationship between the amplitude of the dorsal root action potential and stimulus intensity is shown in Fig. 3 (data from five experiments; the three middle curves show the relationship most frequently encountered, and the other two show extreme variations). The stimulus threshold was determined from the appearance of potential variations resulting from the excitation of fibers with a conduction velocity of 100-125 m/sec (fibers of Group I). As the stimulus intensity increased, this potential variation rapidly grew in size. At a stimulus intensity of 1.2 times the threshold intensity, the height of these potentials was usually 30 per cent of the maximum height (in individual experiments, from 20 to 50 per cent). At the same time, fibers with lower conduction velocities were brought into action, as shown by the broadening of the foot of the peak, which included action potentials being propagated with a velocity of 80–60 m/sec. In most experiments the amplitude of the first peak reached a maximum at stimulus intensities of 1.8–2 times threshold intensity. At these and greater stimulus intensities, all the fibers in Group I were active.

At stimulus intensities as low as 1.2–1.3 times threshold, a second peak appeared that was barely perceptible, which corresponded to velocities of 70-40/m sec and represented fibers of Group II. But up to a certain stimulus intensity, the amplitude of this peak remained low. At a stimulus intensity of 1.8-2 times threshold, the amplitude and areas of the second peak increased sharply. The increase in amplitude was primarily due to impulses traveling with a velocity of 50-40 m/sec. With further increases in stimulus intensity, several more peaks (from two to six) appeared on the screen. At intensities three times threshold, those fibers which had conduction velocities greater than 45-26 m/sec (in different experiments) were active; at stimulus intensities of five times threshold, fibers with conduction velocities of 30-20 m/sec were active.

Thus, stimulation of the central ends of nerves to the quadriceps femoris, at high frequencies (100 per second), in decerebrate regidity, causes contraction of the remaining heads of this muscle. Spread of excitation to the centers for close synergists may occur in response to single stimuli [11], but it is far more pronounced in response to rhythmic stimulation [5, 15]. In form and duration, the observed contractions correspond to the muscle contractions that determine body position, and they are a manifestation of the static stretch reflex. Such reflexes do not develop under conditions where static reflexes are absent in response to

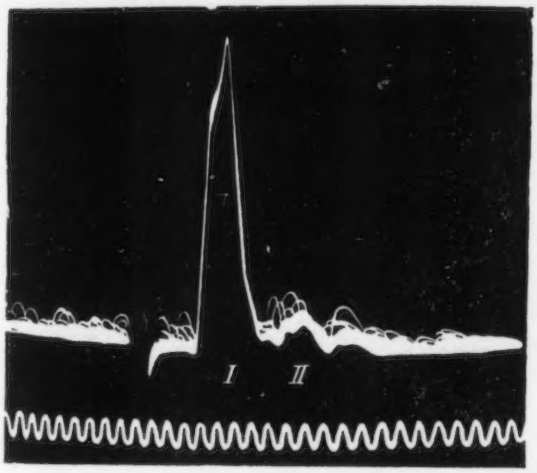

Fig. 2. Action potentials from the sixth lumbar dorsal root upon stimulation of the nerve to the deep and medial heads of the quadriceps. Stimulus intensity 4.5 times threshold. Frequency 100 per second. Distance between stimulating and recording electrodes 86 mm. Calibration 1 mv; time calibration 5000 c/s. I) first peak; II) second peak.

adequate stimuli [3, 12, 13]. Contractions of the quadriceps were observed following comparatively weak stimulation of the muscle nerves.

A comparison of the data obtained from recording of dorsal root action potentials and of reflex responses shows that muscle contractions occur principally in connection with the excitation of afferent fibers having the highest conduction velocities (90-125 m/sec), i.e., the fibers of the primary endings of the muscle spindles [6, 16]. Most of these fibers are active at a stimulus intensity of 1.5-1.7 times threshold intensity (Group Ia) [10]. But an increase in the height of contractions also occurs when stimulus intensities increase to values of 1.8-2 times threshold, where all the fibers of Group I are active. Proprioceptive inhibition in this instance is either weak or completely absent, although the fibers of the tendon receptors are known to be active (Group Ib).

The inhibition of quadriceps tone which was observed upon stimulation of muscle nerves was analogous to the inhibition which occurred when muscle stretch was increased. It was observed most frequently when the intensity of stimulation applied to the muscle nerve was sufficient (more than twice threshold) to excite a large number of afferent fibers in Group II (conduction velocity 70-35 m/sec). These are the fibers of the secondary endings of the muscle spindles, which are stretch receptors like the primary endings [6, 16]. These data indicate that impulses from the secondary endings are the principal factors producing inhibition when the muscle is lengthened. We have been unable to confirm the view that stimulation of tendon receptors plays the decisive role in the origin of stable autogenetic inhibition [4, 14].

It must be emphasized that in our experiments we stimulated not only afferent fibers, but also efferent fibers of muscle nerves, which, according to available published data [2], can inhibit motoneurons of the same muscle via reverse collaterals. At the present time, the character of such effects is not sufficiently understood. According to the data of P. G. Kostyuk [3], reverse collaterals are few

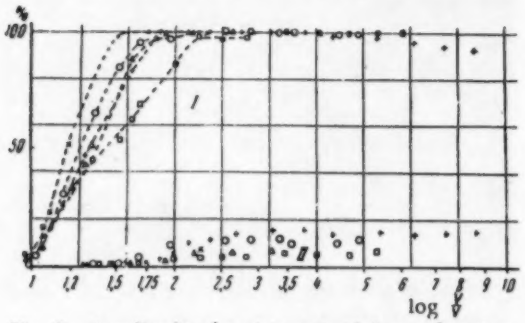

Fig. 3. Amplitude of action potential as a function of stimulus intensity. Abscissa) relative stimulus intensity (logarithmic scale); ordinate) amplitude of action potentials (maximum height of first peak taken as 100%). I) amplitude of first peak; II) amplitude of second peak. Data from five experiments shown (different symbols).

in number. Although theparticipation of antidromic impulses has not been completely excluded, the probability that strong inhibitory influences spread not only to "their own," but also to an extensive field of synergistic motoneurons via reverse collaterals is not very great.

Reflexes in response to stimulation of muscle nerves display wide variability, similar to that which is seen with adequate stimulation of proprioceptors [1]. In individual animals, we observed substantial differences in the character of reflex excitation and inhibition accompanying stimulation of the same nerve. We must suppose that these characteristics of reflex activity in individual animals depend to a large extent on facilitatory and inhibitory influences from the medulla.

SUMMARY

Experiments were performed on cats in decerbrate rigidity. Stimulation of the central ends of some nerves to the quadriceps femoris muscle (100 stimuli per second) evoked a steady contraction or relaxation of the other heads of this muscle. Contractions corresponding to the myotatic reflex are observed with comparatively weak stimuli. Contractions appeared and increased in amplitude in response to stimulation of afferent fibers of Group I (with stimulus intensities up to 1.8-2 times threshold intensity, and with conduction velocities of 70 m/sec and above). Proprioceptive inhibition developed with relatively strong stimuli (more than twice threshold intensity), and was connected with activity of afferent fibers of Group II (conduction velocity of 70-35 m/sec), although the possible importance of antidromic impulses in efferent fibers is not completely excluded. The idea that tendon receptors are of decisive importance in the origin of autogenetic inhibition has not been confirmed. Substantial differences are displayed in the character of reflex excitation and inhibition in individual animals.

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THE EFFECT OF STIMULATION OF GASTRIC MECHANORECEPTORS ON CONDITIONED MOTOR ACTIVITY IN RATS

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In the past ten years various aspects of the problem of interoception have been subjected to intensive experimental investigation. In spite of this fact, very little is known about the role of interoceptive signalling in the formation of an animal's behavior.

Through his studies of cerebral cortical regulation of processes that occur in the internal organs, K. M. Bykov [2] arrived at the conclusion that "functional interoceptive fields created in the cortex are sometimes powerful factors in the formation of behavior."

V. N. Chernigovskii [5] showed that under certain conditions, interoceptive impulses exert excitatory and inhibitory effects on the activity of the skeletal musculature, which is the effector of any behavioral act. In addition, these authors have established that the extent to which interoceptive impulses affect the musculature depends on the state of the organism's internal environment.

In studying the effect of interoceptive stimulation on the act of eating, I. P. Bel'skaya [1] showed that this activity is depressed when the stomach fills. Moreover, this depression depends, not on the weight of the stomach contents, but on the extent to which the walls of the stomach are stretched.

Janowitz and Grossman [6] also observed a change in food intake in dogs upon insertion of food or an indifferent object (a rubber balloon) into the stomach through a fistula.

M. A. Usievich [4] indicated that over-all alimentary behavior shows a definite dependence on the extent of gastric stretching by food or a rubber bag.

A. M. Ugolev and V. N. Chernigovskii [3] showed in experiments on rats that the receptors of the alimentary canal mainly determine the feeding rhythm, and to a lesser extent, the 24-hour consumption of food and fluids.

But as far as we know, no one has yet made a separate study of the influence of the interoceptors on the motor reaction of animals under free-behavior conditions. The purpose of the present study was to investigate the effect of interoceptive stimulation of the stomach on alimentary motor conditioned reflexes in rats.

METHODS

Motor conditioned reflexes were developed in white rats, weighing from 120 to 160 g, in a cage with two feeding trays (see figure).

As may be seen from the figure, the cage is a T-shaped maze in which there are two feeding trays (B and C) and a starting point (A), where the rat stays in the intervals between applications of the conditioned stimuli. Pieces of cooked meat were used as unconditioned stimuli, and were placed in feeding trays B and C. The conditioned stimulus was a flashing light or the sound of a buzzer.

In the initial stages of the development of differentiation between the two sides of the cage on the basis of reinforcement, we observed a positive reaction to the application of both the buzzer and the light in the direction of either feeding tray, independently of the quality of the conditioned stimulus. But since the motor conditioned reflex was reinforced only on one side at the sound of the buzzer and only on the other when the light was flashing, the rats subsequently developed motor differentiation of the sides of reinforcement: The sound of the buzzer was accompanied by a positive motor reaction toward the left feeding tray, and the flashing light by a positive reaction toward the right tray. After eating the food, the rat returned to the starting point in the maze (point A).

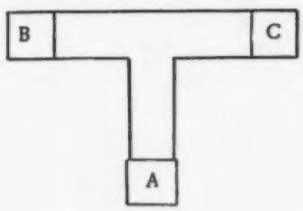

Diagram of cage with two feeding trays (B and C), for producing motor conditioned reflexes in rats.

TABLE 1. Production of a Motor Conditioned Reflex and of Differentiation of the Sides of Reinforcement.

No. of rat	Development of motor conditioned reflex (number of trials)	Development of differentiation of sides of reinforcement (number of trials)	Wrong choices (%).
1	16—18	35—38	10
2	14—15	29—30	5
3	15—16	32—33	10
4	17—18	35—37	10
5	12—14	40—42	5
6	19—20	47—50	10
7	14—16	30—32	10

Mechanoreceptors were stimulated by stretching the walls of the stomach with a rubber balloon. Gastric fistulas had previously been created in these rats, and a small rubber balloon was later inserted through the fistula.

After stable differentiation of the sides of reinforcement had developed, a series of experiments involving stimulation of the gastric mechanoreceptors was carried out. The feeding activity of the animals was tested before each experiment. Experiments were performed only when the animal readily ate the pieces of meat. During the experiment the animal was in the maze with the inflated balloon in its stomach; then the balloon was removed, and after 10-15 minutes the motor alimentary reaction was tested again.

In order to answer several questions that arose in the course of the work, a series of experiments was carried out on rats that were first vagotomized subdiaphragmatically.

RESULTS

In the majority of the rats, a motor conditioned reflex appeared after 14-16 trials and became more stable after 25-27 trials. The rate at which differentiation developed was different in different rats. After stable differentiation had developed, the rats were sometimes observed to run to the wrong feeding tray, but the percentage of wrong choices of the side where reinforcement would occur was not greater than 10. Table 1 shows the results of experiments on the production of conditioned reflexes and differentiation in seven rats that were subsequently used for studies of the effects of gastric mechanoreceptors on motor alimentary activity.

Experiments conducted while the wall of the stomach was distended by the balloon showed that the percentage of wrong choices did not change greatly—i.e., differentiation of the sides of reinforcement was not disturbed. It should be pointed out that at the end of the experiment, 30-40 minutes after insertion of the balloon into the stomach, the rats were less active: they returned to the starting point without ever reaching the food. This lessening of activity could not be explained as the result of fatigue during the experiment, since rats in which the balloon was not inserted were active throughout the entire experiment. Thus, in experiments where the gastric mechanoreceptors were stimulated, all seven rats consistently refused to eat: When they came close to the food, they suddenly returned to the starting point; whereas 15-20 minutes after the balloon was removed, they again readily devoured the pieces of cooked meat.

We may conclude that the application of the conditioned signal against the background of inflation of the balloon in the stomach does not materially alter conditioned-reflex activity: The conditioned motor reflexes and differentiation of the sides of reinforcement are retained. But it is interesting that the animals reject food that is offered.

We may suppose that stimulation of gastric mechanoreceptors for 30-40 minutes is accompanied by a reduction of alimentary excitability, and the animals' reaction outwardly resembles the behavior of animals in a satiated condition.

Insertion of a balloon into the stomach cavity has other effects besides stimulation of gastric mechanoreceptors. We have not ruled out the possibility that other effects besides those directly related to stimulation of these receptors are taking place. To test this, we ran a series of experiments with subdiaphragmatic vagotomy.

Six rats received preliminary subdiaphragamatic vagotomies. As in the previous series of experiments, conditioned motor reflexes and differentiation of the sides of reinforcement were developed in these rats.

In the series of experiments involving stimulation of gastric mechanoreceptors, it was found that differentiation of the sides of reinforcement was not greatly disturbed—i. e., the percentage of wrong choices did not increase. But in the vagotomized rats, stimulation of the gastric mechanoreceptors by inflating the rubber balloon did not result in lowering the animals' appetite for food. Whereas distention of the stomach wall causes unoperated rats to refuse to eat, the same stimulus applied to operated rats is accompanied by active ingestion of cooked meat.

In another series of experiments on five rats, conditioned motor reflexes were developed, and also stable differentiation of the sides of reinforcement. In this case, distention of the stomach by the balloon was accompanied

TABLE 2. Feeding Behavior and Conditioned Motor Reflexes in Rats Before and After Subdiaphragmatic Vagotomy

No. of rat	Before denervation of stomach		After denervation of stomach	
	wrong choices (%)	feeding behavior with stomach distended	wrong choices (%)	feeding behavior with stomach distended
24	10	Refused to eat	10	Ate actively
25	10	The same	10	Ate actively
26	10	The same	15	Ate actively
27	10	The same	10	Ate listlessly
28	5	The same	10	Ate actively

by complete refusal to eat, but the percentage of wrong choices was not greater than 10.

After these figures had been obtained, the rats were subjected to subdiaphragmatic vagotomy – i. e., vagotomy was performed after conditioned reflexes and differentiation were developed.

As is evident from Table 2, experiments carried out 4-5 days after operation showed that while the inflated balloon is in the stomach, conditioned reflex activity is essentially unaltered. These rats ate the food presented, and returned to the starting point in the cage.

SUMMARY

This paper deals with studies of the effect of stimulation of gastric mechanoreceptors on components of the complex conditioned motor food reflex. It was demonstrated that not all components of this reaction were disturbed by stimulation of gastric mechanoreceptors, but only those associated with the act of food ingestion. This indicates that the influence of the mechanoreceptors is not diffuse, but specialized. It was shown experimentally that higher nervous activity was not appreciably changed in the rats investigated, whereas the act of ingestion itself was inhibited in all these animals.

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ROLE OF THE VAGUS NERVE IN THE SECOND PHASE OF GASTRIC SECRETION

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Pavlov and Shumova-Simanovskaya, in 1890 [3], were the first to demonstrate that section of the vagus nerves causes reduction of tonus of the stomach wall and cessation of secretion initiated by sham feeding. Stimulation of the peripheral parts of the sectioned nerves, however, causes some secretion of gastric juice only under certain conditions. Other investigators [4, 5, 6] have also made such observations. Almost all reports have concerned the role of the vagus nerve in the first phase of gastric secretion. As far as its importance for the second phase is concerned, this has up to the present remained insufficiently studied. The reason for this is probably that the method used in these studies, section of the vagus nerve, does not always provide conclusive information. Thus, for example, complete exclusion of the vagi by means of cervical section may cause localized irreversible changes, and these changes may be responsible for general organic dysfunction. Nevertheless, when the nerve is sectioned below the diaphragm, on the wall of the same stomach attached to a Heidenhain pouch, complete exclusion of vagal influence is also not assured. The problem is not resolved by methods involving a subcutaneous isolated pouch (Ivy [8]), since in this case not only is the vagus nerve sectioned, but there is complete denervation and considerable reconstruction of the blood supply.

A method which has slowly gained importance in studies of the vagus nerve in the second phase of gastric secretion is that of transient conduction block of the vagus by means of various pharmacological substances: cocaine, novacaine, nupercaine. Using this technique, investigators have introduced these substances either into the region of the neck where the vagus is situated [2], or into a cutaneous flap into which the truck of the vagus is previously sewn [1]. However, this method does not exclude accessory effects of the applied substance and, consequently, does not provide a solution to the problem. Transient conduction block of the vagus in dogs was accomplished by Anichkov and Kartashevskii [7] after sewing one vagus trunk into a cutaneous sleeve on the neck and cutting the other. Conduction block was produced by either introduction into the sleeve of 0.5 ml cocaine (1%), or by a piece of ice which produced hypothermia.

After studying the literature related to the problem, we evaluated this method as being the most acceptable, and decided to conduct observations under analogous conditions, excluding as far as possible any supplementary effects on other organs or on the organism as a whole. We decided to use mechanical constriction of the cuff, as it was found that during moderate constriction nerve block was completely adequate and stable, and that after constriction was discontinued, conduction was rapidly restored.

METHODS

Experiments were conducted on 4 dogs. One vagus trunk was cut and the other was enclosed in a cutaneous cuff on the neck. Besides this, dog No. 1 had an esophagotomy and stomach fistula, dog No. 2—stomach fistula and isolated fundic Pavlovian pouch, dog No. 3—stomach fistula and isolated fundic Heidenhain pouch and, finally, dog No. 4—stomach fistula, gastroenterostomy and isolated and exposed subcutaneous pouch, formed from the pyloric part of the stomach and the initial part of the duodenal intestine.

Constriction was produced with the aid of a rubber balloon inserted in a metal ring surrounding the cuff. Nerve block resulted when pressure in the cuff was slightly greater than systolic blood pressure, i.e., somewhat greater than 120 mm Hg. It was found that nerve block was maintained even after subsequent pressure reduction from 120-130 mm to 30 mm Hg or less. In connection with this, it can be assumed that when block is produced by pressure it is most probably a stable phenomenon.

RESULTS

During compression of the cuff the Horner syndrome was most prominent, i.e., miosis and ptosis of the nictatating membrane was observed, but also an ipsilateral increase in cephalic skin temperature. This syndrome, associated with conduction failure in sympathetic nerves, served to indicate the onset of block. Concurrently, an abrupt increase in pulse rate to 180-200 per min and changes in respiration (increased depth with same rate) were observed. These changes were apparently a consequence of conduction disorder of the vagus nerve. Both

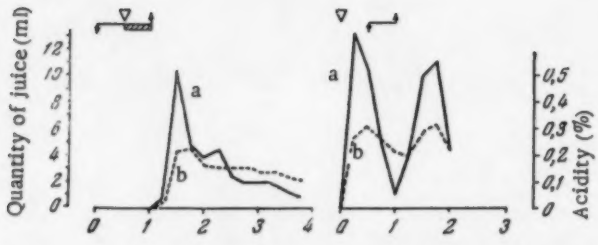

Fig. 1. Changes in stomach secretion in dog No. 1 (fistula) with sham feeding. A) Quantity of juice, in ml; B) Acidity of juice in per cent (free HCl). Small arrows indicate beginning and end of cuff compression; triangles indicate beginning of sham feeding.

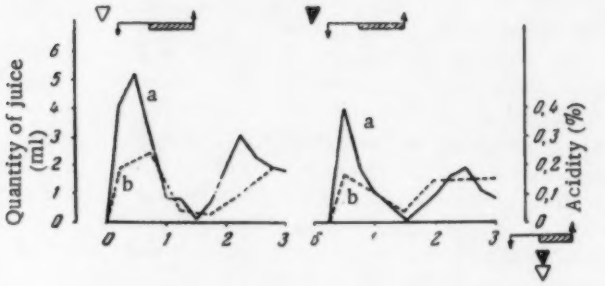

Fig. 2. Changes in secretion of Pavlov pouch in dog No. 2. Triangles indicate introduction into pouch of 100 g meat (solid triangle) and 200 g bread (open triangle). Remaining marks same as in Fig. 1.

phenomena (Horner syndrome and pulse and respiration changes) did not occur immediately after the beginning of compression, but only after 25-30 min. Besides this, with some dogs, particularly when frequent repetitive compression of the cuff was involved, salivation, deglutition, sometimes nausea and emesis were observed. In these cases, following the end of compression and restoration of nerve conduction, it was sometimes possible to observe the appearance of transient (30 min) gastric secretion.

This could be avoided by introducing 1-2 ml novacaine (2%) into the subcutaneous sleeve before the onset of compression. Control studies showed that this dose did not disturb conduction in the vagus trunk, and consequently, did not have any effect on stomach function.

Nerve conduction was restored 3-5 min after the end of compression; the Horner syndrome disappeared and cardiac and respiratory activity returned to the initial level.

The major analysis concerned the relation between the state of the vagus nucleus and alimentary neural stimulation. Experiments were carried out on esophagotomized dogs and others which were fed sham food (broth with added pieces of meat) which freely fell out from the open fistula.

The experiments confirmed the work of Zemskaya [2], who observed in esophagotomized dogs (with one vagus sectioned and the other included in a cutaneous flap on the neck) absence of gastric secretion during sham feeding following the administration into the flap of 1-2 ml nupercaine (0.1%). In our experiments it was found that gastric secretion was absent during 5 min of sham feeding with vagus nerve block, and that secretion might appear only after the end of compression, even if this had occurred for 30 min or more after feeding. Cuff compression halted secretion already initiated, but decompression restored it (Fig. 1).

A similar picture was also observed in dogs with resection of the pyloric region of the stomach and the initial part of the duodenal intestine.

Further experiments were devoted to the problem of the role of the vagus in the second phase of gastric secretion.

Experiments showed that disorder of physiological conduction of the trunk of the vagus resulted in abrupt reduction or complete cessation of secretion (Pavlov or Heidenhain pouch; stomach fistula) which ordinarily appeared following the introduction of food into the stomach cavity (100g meat or 200 g bread, or 1000 ml broth) or mechical stimulation (rubber chain). Nerve conduction disorder also completely prevented and halted secretion which ordinarily was obtained from a fistula of the greater stomach

as a result of stimulation of isolated pyloric pouch mucous membrane. The stimulation in this case was the introduction of a rubber balloon or, for more intense stimulation, a canoe-shaped rye biscuit into the pouch cavity (Fig. 2 and 3).

Absence of fundic gland secretion was observed during the introduction of 500 ml alcohol (5%) into the stomach; considerable depression of secretion was noted during the introduction of 200 ml of the same alcohol into the rectum.

Finally, during transient conduction disorder of the trunk of the vagus (with the other sectioned), we observed not only suppression of secretion but also of stomach motor activity. In these cases, introduction of a balloon into the stomach cavity caused only insignificant contractions of the stomach wall. This was noted even with a highly inflated balloon.

On the basis of these results it was possible to assume that suppression of secretion and motor activity of the stomach in connection with conduction disorder of the trunk of the vagus nerve in the neck is related not only to exclusion of secretory, motoric and tonic effects of this nerve, but also to an intensification of the influence of the sympathetic nervous system both on the tonus of stomach vessels and, possibly, on its function.

Special experiments, however, showed that an increase in general sympathetic tonus and concomitant augmentation of blood adrenalin apparently does not occur. The evidence concerning this was the absence of any change in the quantity of blood sugar (Hagedorn-Jensen method) both at the onset of nerve block and after its release (Table).

According to our experiments, secretory impulses appear in the vagus nucleus throughout the duration of protected sham feeding (apparently, for the total period of secretion), and are not limited to only the onset of feeding. Disorder of physiological conduction in the vagus nerve excludes not only the first, but also the second phase of gastric secretion, and secretion which normally appears in connection with stimulation of the mucous membrane of an isolated pyloric gland. In addition, secretion of the

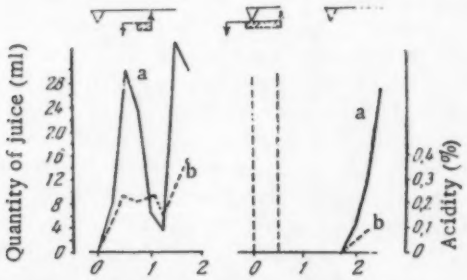

Fig. 3. Changes in stomach secretion in dog No. 4 (fistula). The triangle and the line show the moment and the duration of stimulation of pyloric pouch mucous membrane by introduction of a rye biscuit. Remaining marks same as in Fig. 1.

TABLE. Quantity of Blood Sugar (in milligrams-percent) (Dog No.3)

Experiment No.	Before exclusion of vagus nerve	At onset of exclusion	After restoration
4	0,075	0,072	0,075
6	0,082	0,082	0,084

fundic gland, produced by introducing 500 ml alcohol (5%) into the stomach or 200 ml of the same solution into the rectum is also inhibited. Throughout all these conditions the quantity of blood sugar remains constant, indicating the absence of any change in general sympathetic tonus or quantity of blood adrenalin.

SUMMARY

This work was conducted on 4 dogs esophagotomy and gastric fistula, isolated Pavlov's pouch, gastric fistula and Heidenhain's pouch, fistula of the stomach and isolated gastric pouch made of pylorus), in which one of the vagus nerves was enclosed into a cuff on the neck, and the other divided. As shown by experiments, the compression of the cuff by a force a little over 120 mm Hg provokes a block of the vagus conductivity, which may be estimated by the acceleration of the pulse and the appearance of Horner's syndrome. This excludes the first and the second phases of the gastric secretion, as well as secretion provoked by stimulating the mucous membrane of the isolated pyloric pouch.

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THE INDEPENDENCE OF MECHANISMS REGULATING THE DIAMETER OF REGIONAL ARTERIES OF THE BRAIN (INTERNAL CAROTID AND VERTEBRAL) AND OF THE PIAL ARTERIES

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Our studies of the function of the regional arteries of the brain (internal carotid and vertebral arteries) under conditions such that the flow of blood out of the brain is disturbed, in traumatic brain edema, and in terminal conditions have shown that these vessels are of great importance in compensating for disorders of cerebral circulation.

It has subsequently proved necessary to determine whether the regional arteries of the brain participate in the development of those changes in cerebral circulation which result from a change in the diameter of the pial arteries, and are connected, in particular, with the functioning of the brain. The answer to this question is very difficult to obtain. It has been shown [10] that in natural sleep the total blood flow to the brain (method of Kety and Schmidt) is not reduced, but even increases; and on the other hand, during intense brain activity (solving problems in arithmetic) the blood supply to the brain does not increase, although there are electroencephalographic changes typical of intensified cortical activity.

In the present investigation, we studied the state of the regional arteries of the brain in those cases where considerable dilatation of the pial arteries supplying the cerebral cortex occurs.

In this case the number of functioning capillaries increases, and dilatation of these capillaries takes place [4]–i.e., the circulation in the brain is unquestionably enhanced. Similar changes in brain circulation occur, on the one hand, during temporary occlusion of the trachea, and on the other, after local application of strychnine to the cerebral cortex. If we record the condition of regional arteries of the brain and that of the pial vessels simultaneously, we will be able to demonstrate the functional connection between the mechanisms that determine the blood flow to the brain as a whole and those that determine the blood supply to small regions of the brain.

METHODS

Experiments were carried out on 16 mature rabbits, anesthetized in some instances with chloral hydrate (0.4 g/kg). After the operation the animals were given heparin (0.3-0.4 ml/kg). The brain circulation was altered either by temporary occlusion of the trachetomy tube or by local application of 0.5-1% strychnine, made isotonic by adding NaCl, to the surface of the brain.

The state of the regional arteries of the brain was determined on the basis of simultaneous recording of arterial pressure in the aorta and the circle of Willis, which allowed us to judge the magnitude of the resistance in the internal carotids and the vertebrals. In some experiments a systemic arterial pressure compensator was employed [3].

The condition of the pial vessels was recorded by serial microphotography at intervals of five or ten seconds, with the time of each photography being indicated on the same kymograph record as the record of arterial pressure. In each frame of the film, the diameter of these vessels was measured with the ocular micrometer of a microscope. When these diameters were subsequently plotted on the kymograph record, it was then possible to obtain an idea both of the state of the regional arteries of the brain and of the time course of changes in the diameter of the pial vessels.

RESULTS

The dilatation of the pial vessels that accompanies occlusion of the trachea is usually regarded as the result of an increase in the carbon dioxide concentration in the blood. Most authors [2, 7] believe that CO_2 is the principal regulator of the cerebral circulation since, by accumulating in the brain tissue when the blood supply is inadequate and producing dilatation of the vessels, it quickly restores the correspondence between the "needs" of the neuronal elements of the brain for an increased blood flow and the magnitude of the flow itself.

The effect of increasing the CO_2 content of the blood (hypercapnia) on the pial arteries has been shown by many authors [2, 8], but the state of the internal carotids and vertebrals under these circumstances has not been determined. Simultaneous recording of the arterial pressure at both ends of the regional arteries of the brain, enabling us to judge the conditions of the lumen of these vessels,

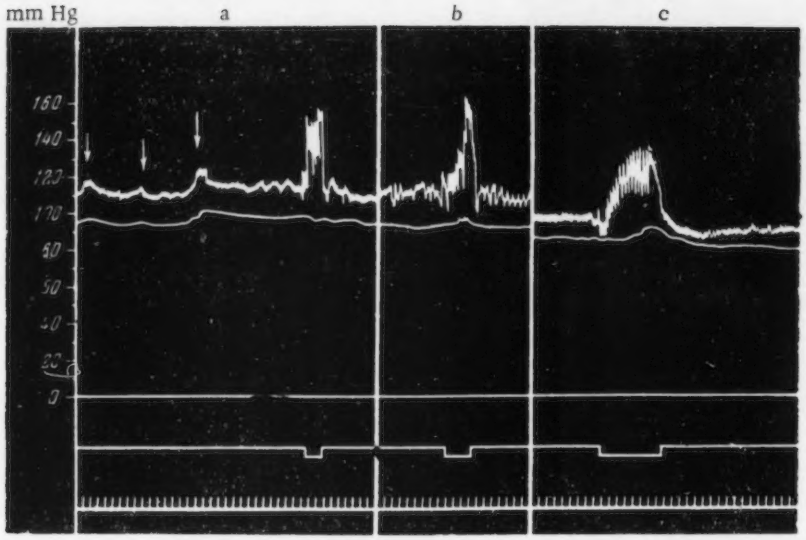

Fig. 1. Changes in arterial pressure in the aorta and in the circle of Willis upon occlusion of the tracheotomy tube at various times. The difference between the indicated pressures gives an expression of the magnitude of the resistance in the regional arteries of the brain (internal carotids and vertebrals). Tracings (top to bottom): arterial pressure in aorta; arterial pressure in circle of Willis; base line; clamping of tracheotomy tube; time (5 sec). Arrows designate times when animal moved, with simultaneous alteration of pressure. a) trachea clamped for 12 seconds; b) trachea clamped for 18 seconds; c) trachea clamped for 45 seconds.

showed that during temporary occlusion of the trachea they behave differently from the pial arteries (Fig. 1). Thus, when the animal moves, even an insignificant elevation of systemic arterial pressure is accompanied by a corresponding change in the pressure in the circle of Willis; whereas when the trachea is clamped, despite a sharp elevation of systemic arterial pressure (the well-known reflex from the chemoreceptors), the blood pressure in the circle of Willis actually falls a little (Fig. 1a). The same thing happens when the tracheotomy tube is clamped for a longer period (Fig. 1b); when the trachea was clamped for 45 seconds the arterial pressure in the circle of Willis rose a relatively small amount, and the rise was delayed (Fig. 1c). From this we may assume that when the trachea is clamped, and during the subsequent hypercapnia and hypoxemia, the regional arteries of the brain not only do not dilate, but actually constrict at the same time as the systematic arterial pressure is elevated. Apparently the internal carotid and vertebral arteries, unlike the cerebral vessels lying peripheral to them, participate in the regulation of systemic arterial pressure.

It is well known that even a slight amount of hypercapnia produces a sharp dilatation of the pial arteries. To determine the diameter of these vessels at the same time as the state of the regional arteries of the brain, we recorded the diameter of pial vessels on photographic film. An example of such an experiment is shown in Fig. 2. Because a systematic arterial pressure compensator was being used, the level of arterial pressure began to oscillate while the trachea was clamped (for approximately 45 sec), but hardly rose at all, while the arterial pressure in the circle of Willis remained constant. At the same time, the pial arteries dilated markedly. Thus, while the trachea is clamped and during the resulting hypercapnia the pial arteries dilate, while the internal carotids and vertebrals actually constrict or keep the same diameter.

In another series of experiments we examined the effect of strychnine on the circulation in a small region of the brain. When this substance is applied locally to the cerebral cortex, convulsive discharges appear in the electrocardiogram, indicating that the activity of cortical neurons is intensified; and dilatation of pial vessels occurs simultaneously [8]. Our studies together with A. I. Roitbak have shown that these changes in the circulation to a small region of the cortex are caused, not by a direct action of strychnine on the wall of the arteries, but by an effect exerted on the arteries by the nerve elements whose activity is markedly increased.

We found (Fig. 3) that local application of strychnine to the cerebral cortex is followed by a considerable dilatation of the pial arteries, whereas the level of arterial pressure in the aorta and the circle of Willis remains unchanged—provided, of course, the strychnine is not applied to the motor cortex and the animal does not become convulsive.

Thus, our data, obtained during tracheal occlusion and during the local action of strychnine on the cortex, provide evidence that the mechanisms whereby the systemic arterial pressure and the diameter of regional arteries of

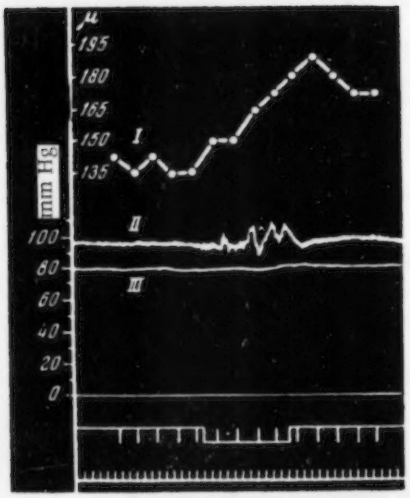

Fig. 2. Changes in the diameter of the pial arteries and in arterial pressure in the aorta and the circle of Willis during temporary occlusion of the tracheotomy tube. Aortic pressure was compensated. I) diameter of pial artery; II) arterial pressure in aorta; III) arterial pressure in circle of Willis. Marker indicates time when trachea was clamped, as well as times when microphotographs of pial vessels were taken; time marker, 5 sec.

the brain are regulated do not participate in the dilatation of cerebral arteries, and therefore can not participate in increasing the local circulation in various regions of the brain; under physiological conditions this local circulation is usually connected with the activity of the nerve elements and with their "need" for a supply of blood.

The peripheral (or local) circulation in all organs possesses a regulatory mechanism that is relatively independent of the central (or systemic) circulation [6]. The mechanisms that regulate the cerebral circulation also show this independence—i.e., the circulation of the blood in the brain can change even when the central circulation, specifically the systemic arterial pressure, remains unchanged [1, 2, and our own studies]. On the other hand, changes in the central circulation may have no effect on the cerebral circulation, since it is common knowledge [9] that when arterial pressure is elevated in hypertension, or lowered in diabetic coma, the cerebral blood flow may not change.

Our studies have shown that in the regulation of cerebral circulation the regional arteries of the brain (internal carotids and vertebrals) are very important, since they compensate for certain disorders of cerebral circulation. It has been established that when the outflow of blood from a region of the brain is disturbed, constriction of the regional arteries of the brain occurs, and this prevents the development of stasis in the brain during the period before the collaterals begin to ensure the outflow of venous blood from the brain. When cerebral edema develops, the regional

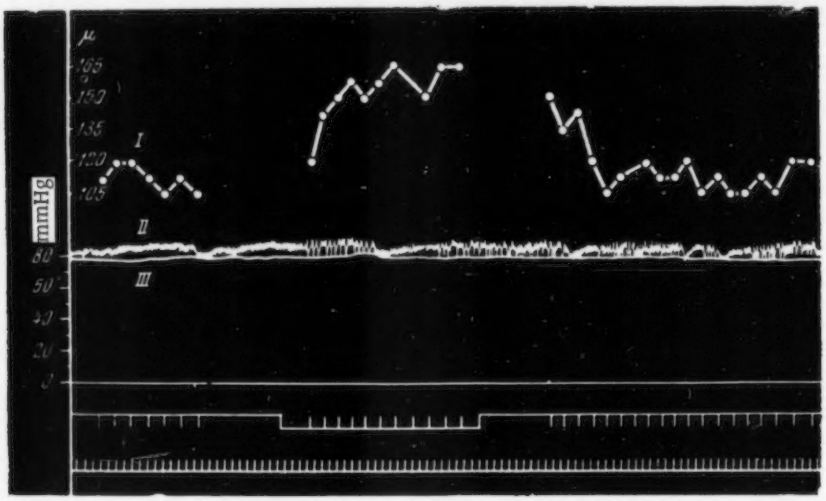

Fig. 3. Changes in the diameter of pial arteries, and in arterial pressure in the aorta and circle of Willis following local application of 0.5% strychnine to the cortex. I) diameter of pial artery; II) arterial pressure in aorta; III) arterial pressure in circle of Willis. Marker indicates time of application of strychnine, as well as times when microphotographs of pial vessels were taken. Time marker, 5 sec.

arteries of the brain constrict, causing a drop in pressure in the brain capillaries, so that the transudation of fluid into the tissue is reduced and the development of edema is retarded. In terminal conditions the regional arteries completely isolate the arterial system of the brain from the other arteries of the organism, so that when the systemic arterial pressure falls to zero, blood continues in the cortex for several minutes.

Under conditions where disorders of the cerebral circulation are compensated, we have in several instances observed a completely coordinated response of the regional arteries of the brain and the pial arteries. Thus, when the outflow of blood from the cranial cavity is disturbed, or in traumatic edema of the brain, when increased flow of blood to the capillaries of the brain may be injurious, the pial arteries constrict along with the regional arteries. In terminal states, after a marked constriction of the regional arteries of the brain, a slow peristaltic wave of contraction develops in the pial arteries, causing blood to flow in the right direction in the brain capillaries after it has stopped in other organs. But in other cases—e.g., when the vital functions of the organism are restored after the animal has passed through the agonal state and clinical death—when brain edema develops and the pial arteries are markedly dilated, the regional arteries of the brain constrict strongly, compensating for the changes mentioned, which would damage the brain.

The experiments described in this paper have shown that the local enhancement of cerebral circulation, caused by an increase in the content of CO_2 in the blood or by intensified functional activity of the cortical neurons, may be brought about through the agency of the pial and other small cerebral arteries without the participation of the internal carotids or the vertebrals.

All of this indicates that two independent mechanisms exist for the regulation of the cerebral circulation. On the one hand, we have the mechanism of the regional arteries of the brain, which determines the intensity of the blood flow to the cerebrum as a whole, and takes an active part in the compensation of certain disorders of cerebral circulation. On the other hand, we have the mechanism of the smaller arteries located on the surface of the brain or inside it, which determine the blood flow to relatively small regions of the central nervous system. The second mechanism is apparently of great importance in those changes of cerebral circulation which are connected with the level of the functional activity of neuronal elements of the brain. We reported on this as far back as 1957 [5].

SUMMARY

The author estimated the resistance in the internal carotid and vertebral arteries from measurements of arterial blood pressure in the aorta and the circle of Willis. These arteries become constricted when the tracheotomy tube is temporarily occluded. Under these conditions the pial arteries may dilate independently of the regional arteries of the brain, while the arterial pressure in the aorta and the circle of Willis remains unchanged. The same changes occur in response to local application of strychnine to the surface of the brain. Thus, the mechanism controlling the diameters of the pial arteries are independent of the mechanism regulating the state of the regional arteries of the brain (internal carotids and vertebrals).

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* See C. B. translation.

THE INFLUENCE OF THE CEREBRAL HEMISPHERES ON THE ADRENOCORTICOTROPIC ACTIVITY OF THE HYPOPHYSIS

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The present studies are based on the view, expressed by I. P. Pavlov, that all the functions of the organism are subject to regulating influences from higher divisions of the central nervous system. The work of B. V. Aleshin and his colleagues [1, 2] has shown that a mechanical influence exerted on the cerebral cortex alters the dependence of the thyroid gland and of sexual functions on the hypophysis.

Our earlier observations made it clear that the production of hormones in the adrenal cortex, and the transformation of these hormones, depend on impulses from the brain [8].

The purpose of the present investigation was to study the effect of a disruption of the normal state of the cerebral cortex on the adrenocorticortropic activity of the hypophysis. To answer this question, we examined the changes in the extent to which adrenocortical androgenic function is shifted in hypophysectomized rats given extracts of the hypophyses under investigation.

METHODS

The donors were male rabbits weighing 2-2.5 kg. The cerebral cortex was treated as follows: Silver plates were placed on the motor, olfactory, parietal, temporal, and occipital cortex along the midline, in such a way that one plate affected both cerebral hemispheres. The temporal lobe was subjected to a one-step operation in which two plates were placed upon the two halves of the cortex. In this way, a constant and prolonged disturbance of the normal condition of the brain was achieved.

Ten days after the operation these rabbits were sacrificed by injecting air into an ear vein; the content of adrenocorticotropic hormone (ACTH) in their hypophyses was then determined. The content of ACTH was determined and bio-assayed by the method of G. Sayers and M. Sayers [9], as modified by us [4], on hypophysectomized rats, 24 hours after hypophysectomy. For this purpose we determined the quantitative change in androgen content in the right adrenal $1\frac{1}{2}$ hours after injection of hypophyseal extract into the abdominal cavity.

The initial androgen content was determined from the left adrenal, which was removed before the extract was injected. The androgen content was determined by the method published by us previously [5], which makes use of the Zimmermann color reaction [10]. The androgen content was expressed in μg-%. Androgens were extracted from donor adrenals for biological testing in the same way as for the colorimetric determination of androgens. This extract was tested on the combs of day-old chicks by the method of Ya. M. Kabak [3].

In previous papers [6, 7] we discussed the effect of the superior cervical ganglia on the adrenocorticotropic function of the hypophysis. The effect of a disturbance of sympathetic activity on the adrenocorticotropic function of the hypophysis was established from the change in the androgen content in the adrenal cortex of hypophysectomized rats that received hypophyseal extract from donors whose superior cervical ganglia had either been removed or stimulated.

The reduction of the androgen content in the adrenal cortex of recipients after the injection of hypophyseal extract from sympathectomized donors, as compared with that observed after injection of hypophyseal extract from intact donors, was interpreted as indicating that the adrenocorticotropic activity of the hypophysis was elevated. On account of the low androgen concentration in the gland, it was assumed that androgen secretion exceeded androgen production in the adrenal cortex. On the other hand, the elevated androgen concentration in the adrenal cortex of recipients of hypophyseal extract from donors whose superior cervical ganglia had been stimulated was interpreted as due to a decrease in the adrenocorticotropic activity of the hypophysis.

But later experiments on rabbits, in which hormone production in the adrenal cortex and hormone secretion into the blood were studied simultaneously, showed that an elevation of the androgen content in this gland is accompanied by an increase in androgen secretion into the blood; when the androgen content in the adrenal cortex is reduced, the androgen level in the blood is also reduced. Thus we suggest that when hypophyseal adrenocorticotropic function is enhanced, not only the production of androgens in the adrenal cortex, but also the secretion of androgens into the blood, is increased.

TABLE 1. Androgen Content (in μg-%) in the Adrenal Cortex of Recipients Before and After Injection of Hypophyseal Extracts From a Normal Animal and From Animals Subjected to Mechanical Pressure on the Cerebral Cortex

No. of animal	Normal			Motor cortex			Olfactory cortex			Parietal cortex			Temporal cortex			Occipital cortex		
	left, before injection	right, after injection	difference from initial level	left, before injection	right, after injection	difference from initial level	left, before injection	right, after injection	difference from initial level	left, before injection	right, after injection	difference from initial level	left, before injection	right, after injection	difference from initial level	left, before injection	right, after injection	difference from initial level
1	150,8	244,4	+62,0	104,0	98,1	—5,7	80,2	69,6	—13,3	127,4	112,0	—12,2	134,7	203,0	+50,0	105,4	175,0	+66,0
2	150,0	212,5	+41,7	88,3	91,9	+4,0	90,9	71,2	—21,0	239,0	180,0	—24,7	103,0	196,0	+90,2	153,3	271,9	+77,4
3	159,9	278,7	+74,3	72,3	77,0	+6,5	85,0	82,0	—3,6	152,9	125,5	— 8,8	58,5	95,0	+60,6	164,9	232,2	+40,8
4	186,6	353,7	+89,0	82,5	78,0	—5,5	112,0	98,3	—12,3	198,0	173,0	—12,7	91,6	149,0	+62,6	139,2	201,6	+44,8
5	198,8	303,3	+52,6	112,6	104,2	—6,6	80,0	72,7	—10,0	213,0	184,3	—13,6	126,0	195,0	+54,7	212,1	286,0	+35,3
6	189,7	272,5	+49,0	98,7	86,7	—12,2	117,0	92,1	—21,3	187,2	158,0	—15,6	118,8	171,3	+44,1	97,8	167,4	+71,1
7	80,2	120,8	+50,0	135,2	151,3	+19,3	98,0	83,6	—14,7	174,1	149,6	—14,1	142,5	198,9	+39,5	222,0	299,7	+35,0
8	107,0	184,0	+72,0	123,6	213,2	—10,0	109,2	101,7	—6,9	108,7	91,0	—16,3	201,1	297,4	+47,9	148,0	215,1	+45,3
9	173,0	299,0	+74,2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean	155,0	252,0	+62,3	116,2	112,5	— 3,2	96,4	82,9	—12,8	175,3	146,8	—16,3	122,2	188,2	+54,0	155,3	231,1	+51,9

TABLE 2. Androgen Content in Adrenal Cortex of Donor Rabbits Before and After Mechanical Pressure on the Cerebral Cortex (in milligrams)

No. of animal	Weight of combs from intact chicks	Normal		Olfactory cortex		Parietal cortex		Temporal cortex		Occipital cortex	
		weight of combs after test of extract	difference from control (%)	weight of combs after test of extract	difference from control (%)	weight of combs after test of extract	difference from control (%)	weight of combs after test of extract	difference from control (%)	weight of combs after test of extract	difference from control (%)
1	12	18	+50,0	8	—55,6	8	—55,6	50	+211,1	45	+150,0
2	13	25	+92,3	14	—44,0	9	—64,0	57	+128,0	46	+84,0
3	10	15	+50,0	6	—59,7	11	—26,7	35	+133,3	34	+126,6
4	11	15	+36,4	11	—27,0	9	—40,0	37	+146,6	38	+153,3
5	13	18	+39,0	10	—45,6	8	—55,6	54	+200,0	39	+116,6
6	14	34	+143,0	19	—45,9	15	—55,9	126	+230,6	73	+114,7
7	13	24	+84,6	9	—59,4	10	—58,4	67	+180,0	50	+108,3
8	10	—	—	—	—	—	—	—	—	—	—
9	12	—	—	—	—	—	—	—	—	—	—
Mean	12,0	21,3	+77,5	11	—48,2	10	—51,1	60,7	+185,0	46,4	+121,0

RESULTS

Examination of hypophyses from intact donors. Adrenocorticotropic extract from the hypophyses of intact donors, when injected into recipients, produces a marked increase in androgen content in the adrenal cortex. Whereas the androgen content in the left adrenal averaged 155 μg-% before injection of the extract, that in the right adrenal after injection of the extract was 252 μg-%—i.e., the androgen content increased 62.3% in relation to the androgen content in the left adrenal. These values, which show the androgen content in the adrenals, were used as a control.

Subsequent observations on the content of ACTH in hypophyses of donors subjected to the application of silver plates on various lobes of the cerebral cortex showed that the disruption of the normal state of the cortex has different effects on the adrenocorticotropic function of the hypophysis, depending on the lobe involved (Table 1).

Application of plates to various regions of the cerebral cortex. Injection of adrenocorticotropic extract from the hypophyses of donors in which the plates were applied to the motor cortex did not cause an elevation of the androgen content in the adrenal cortex. Prior to the administration of extract, the androgen content in the left adrenal averaged 116.2 μg-%, and after injection the androgen content in the right adrenal was approximately the same (112.5μg-%), which is a difference of only 3.2% from the initial level. A comparison of these results shows that ACTH was practically absent from this extract. Thus, when the agent is applied to the motor cortex the result is a distinct reduction in the adrenocorticotropic activity of the hypophysis.

Similar results were obtained after injection of extracts from donors in which the plates were applied to the olfactory cortex. Thus, whereas the androgen content in the left adrenal before injection of the extract was 96.4 μg-%, on the average, after injection the androgen content in the right adrenal was still 82.8μg-%. Consequently, the androgen content in the right adrenal was reduced to 12.8% below the initial level.

The same result was observed following injection of hypophyseal extracts from donors in which the plates had been applied to the parietal cortex. Before the injection of extract, the androgen content in the left adrenal averaged 175.3 μg-%, and after injection the androgen content in the right adrenal remained at 146.8 μg-%, i.e., the androgen content was reduced 16.3% below the initial level.

The results of these three series of experiments demonstrate that adrenocorticotropic extract from hypophyses of donors subjected to the application of plates on the motor, olfactory, and parietal cortex not only does not cause the androgen content in the adrenal cortex of recipients to increase in relation to the controls, but even reduces the androgen level somewhat in relation to the initial level; this can be regarded as an indication that adrenocorticotropic activity has been reduced.

Hypophyseal extract from donors in which the plates were applied to the temporal and occipital lobes produced a noticeable increase in the androgen content in the adrenal

cortex of the recipients. The results of these studies showed that for the temporal-cortex group, whereas the androgen content in the left adrenal averaged 115.3 μg-% before injection, injection of hypophyseal extract from the donors of this group caused the androgen content of the right adrenal to increase to 231.1 μg-%, an increase of 51.9%. Thus, in this instance adrenocorticotropic activity did not undergo noticeable changes in comparison with the controls. The same result was obtained following injection of hypophyseal extract from donors of the occipital-cortex group. In the left adrenal prior to the injection of extract the androgen content was 122.2 μg-%, and in the right adrenal after injection it was 188.2 μg-%, i.e., the androgen content increased 54.9% in comparison with the initial level.

The values obtained in these two series of experiments show that the ACTH content does not change in relation to the control.

Investigation of androgens in adrenals of donors by means of comb growth in chicks. Tests of adrenal extract from intact donors resulted in a distinct increase in the weight of combs, which is evidence that the androgen content in these adrenals is elevated. The weight of combs from intact chicks averaged 12 mg, and in tests of androgenic extract from the adrenals of intact donors the weight of the combs increased to 21.3 mg, on the average—an increase of 77.5%. The weights of combs from chicks that were given adrenal extract from intact donors served as a control.

The results of the colorimetric determination showed that under the influence of ACTH contained in the hypophysis of intact donors, the androgen level in the adrenal cortex of recipients increased sharply: 62.3%, on the average. We found approximately the same increase in the weight of combs when we used extract from the adrenals of intact donors. On the other hand, extract from the adrenal cortex of donors in which plates were applied to the olfactory and parietal lobes caused no noticeable changes (11-10 mg, on the average) in the weight of combs. We may conclude that the adrenals of these donors contained practically no androgen (Table 2).

But adrenal extract from donors in which plates were applied to the temporal lobe increased comb weight to 46.4 mg, or 121%, in comparison with the control. Following application of plates to the occipital lobe, adrenal extract from the donors produced a still more pronounced increase in comb weight (60.7 mg). These results show that under these circumstances the androgen content in the adrenals is elevated in relation to the control (see Table 2).

Our observations on the changes in adrogen content in the adrenals of recipients and donors, presented in Tables 1 and 2, show that disruption of the normal state of different regions of the cerebral cortex has different effects on the processes of androgen formation in the adrenals.

SUMMARY

The author studied the effect of a disturbance in the normal state of the cerebral cortex on adrenocorticotropic activity of the hypophysis in rabbits. Shifts in the adrenocorticotropic activity of the hypophysis as a result of injury to the cerebral cortex were determined from changes in the androgen content in the adrenal cortex of hypophysectomized rats given extracts of these hypophyses. It was established that when silver plates are applied to the anterior part of the cortex of both hemispheres, the androgen content is diminished in the adrenal cortices of both donors and recipients; this may be regarded as an indication that the adrenocorticotropic activity of the hypophysis is reduced.

Conversely, the high androgen content in the adrenal cortex of hypophyseal extract donors and recipients following application of silver plates to the posterior cerebral cortex of donors (in comparison with the effect of hypophyseal extracts from intact animals) demonstrates that in this case the adrenocorticotropic activity of the hypophysis does not undergo substantial changes.

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CHANGES IN RESPIRATION FOLLOWING VOLUNTARY HYPERVENTILATION

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It has been established that the changes in respiration that occur following hyperventilation are caused by a complex of different factors. These include both neuroreflex and humoral influences. A detailed survey of the respiratory changes produced by hyperventilation can be found in the literature [1, 2, 3, 7, 8].

In the present investigation we have studied the effect of hyperventilation on subsequent respiration in athletes in different stages of training.

METHODS AND RESULTS

The usual pneumographic methods were used in these experiments. We examined 148 athletes having various specialties and skills, as well as 26 persons not engaged in athletics.

Experiments with repeated tests of hyperventilation for various periods (10, 25, and 40 inspirations and expirations, each having a volume of 70-83% of vital capacity, for 20, 50, and 80 seconds) showed that the character of the respiratory changes that ensue in athletes does not depend on the time of day (experiments were carried out between 7 AM and 11 PM), and does not change over a period of 3-4 days.

Experiments with these tests on 68 athletes in different stages of training gave the following results. In 35 subjects the character of respiration before, during, and after hyperventilation was comparatively uniform, both when they were in poor training and when they were in excellent training. In the other athletes, various changes were seen. Thus, during the period of hyperventilation in 25 persons at an early stage of training, we noted interrupted respiratory cycles and a variable amplitude, which decreased toward the end of the test. In the same individuals, changes in amplitude and arrythmia of respiratory movements were observed after hyperventilation, as well as periodic breathing. When these individuals reached excellent condition, the phenomena just described either stopped or became less pronounced. In eight athletes, hyperventilation produced inhibition of the activity of the respiratory center in an early stage of training, which was replaced by elevation of the activity of this center when the subjects were in condition (Fig. 1).

In the great majority of the athletes, we failed to observe apnea after hyperventilation.

In the light of this fact we examined 26 persons not engaged in sports. No noticeable differences were found between the character of post-ventilation breathing in athletes and nonathletes. Various types were found in both groups; the most characteristic of these are shown in Fig. 2.

The first type is distinguished by the fact that hyperventilation has no noticeable effect on respiration. The characteristic of the second type is an increase in the rate and depth of respiration after hyperventilation, as compared with the initial state. The third type characteristically develops periodic respiration, and the fourth type, prolonged apnea.

The first two types and their varieties were encountered among 76% of the subjects (athletes and nonathletes). The last two types were found in 24% of the subjects, with apnea after hyperventilation being observed in some individuals only at an early stage of training. "Pure" apnea was observed in only eight persons, two of whom had studied physiology and knew that hyperventilation "is invariably followed by apnea." Apnea was noted after hyperventilation of 10 or more respiratory cycles. The duration of the apnea was found to be directly related to the duration of hyperventilation, which is in agreement with available data [5].

We conducted separate experiments with 18 students who were given the following six tasks at intervals of 3-6 minutes: 1) a bell rang (50-120 seconds), and the subject was asked to determine how long it rang; 2) the subject was ordered to read 1-1½ pages of a popular text in 60-80 seconds and recall correctly what he had read; 3) hyperventilation was performed at the command of the experimenter (25 cycles in 50 seconds); 4) the task was the same as the previous one, but the first task given at the end of hyperventilation; 5) the same, but followed by the second task; 6) control hyperventilation.

The first and second tasks usually had no noticeable effect on respiration (Fig. 3). After the third task, respiration either increased in intensity or remained essentially unchanged, in most cases.

During the fourth and fifth tasks, the first and second indifferent stimuli usually had an inhibitory effect on respiration. We observed this in 10 out of 18 subjects. In

* Deceased.

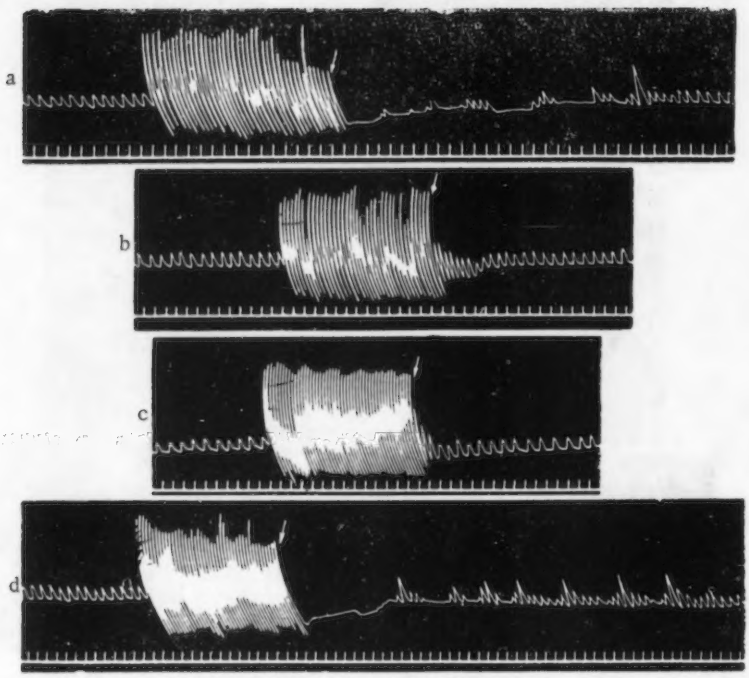

Fig. 1. Effect of hyperventilation on respiratory changes in athletes at various stages of training. Pneumograms recorded: a) in March, 1952 (early stage of training); b) in December, 1952 (halfway through training); c) in April, 1953 (in shape for competition); d) in September, 1953 (out of training). Interpretation of records (from top down): pneumogram; time (5 sec); (subject St-v, athlete of the first class, light heavyweight, 20 years old).

four subjects, respiration was inhibited during only one of the tasks (the fourth or fifth); and in four others, the character of respiration after hyperventilation during the fourth and fifth tasks was no different from that during the third and sixth tasks.

Our data permit us to suppose that in the complex network of nervous and humoral factors under whose influence the reaction of the respiratory center to hyperventilation develops, cortical influences are of great importance.

Excitation that has developed in the motor zone of the cortex during voluntary hyperventilation, upon irradiating to subcortical nuclei and the brain stem, can in many instances cause the rate and depth of respiration to increase for a period of time, as we and other authors have observed [1, 6, 9]. It ought to be noted particularly that in this case the rate of respiration is often close to the rate of respiration in hyperventilation. These observations can be explained by an Ukhtomskii-type assimilation of rhythm (the possibility of assimilation of rhythm by the respiratory center has been shown by E. B. Sologub [4]).

Cortical influences on respiration after hyperventilation are evidently the controlling factors, since in 365 out of 593 cases the rate of respiration increased after hyperventilation (in 228 cases it remained unchanged or became somewhat slower, or the third and fourth types of respiration were observed).

The inhibitory effect of humoral factors became dominant during the execution of the fourth and fifth tasks. In these cases, normally indifferent agents apparently created new foci of excitation in the cortex, inhibiting cortical stimulation of the medullary respiratory center, and the humoral factors came into their own.

SUMMARY

The following types of respiration were observed in 148 athletes and 26 nonathletes after voluntary hyperventilation: 1) ordinary respiration; 2) respiration of increased rate and depth; 3) periodic respiration; and 4) apnea. The first two types were seen in 76% of the persons examined, and the last two in 24%. The incidence of the first two types increases in athletes as training progresses.

The changes that occur in respiration after hyperventilation are caused by a complex of nervous and humoral factors that result in enhanced respiration.

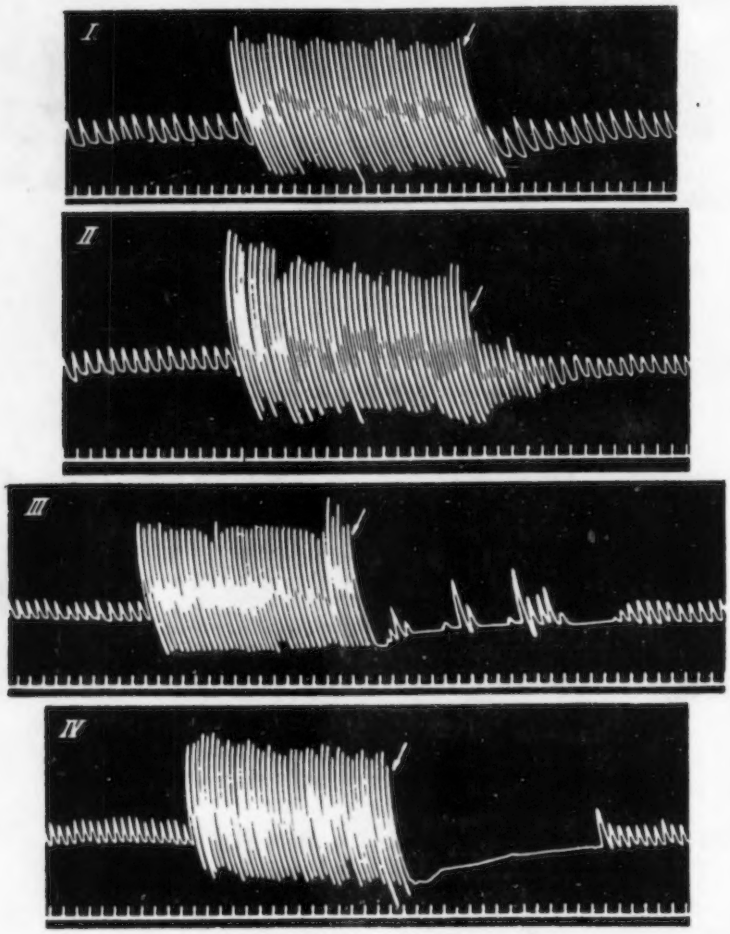

Fig. 2. Various types (I, II, III, IV) of respiratory change following hyperventilation. Interpretation of curves is the same as in Fig. 1.

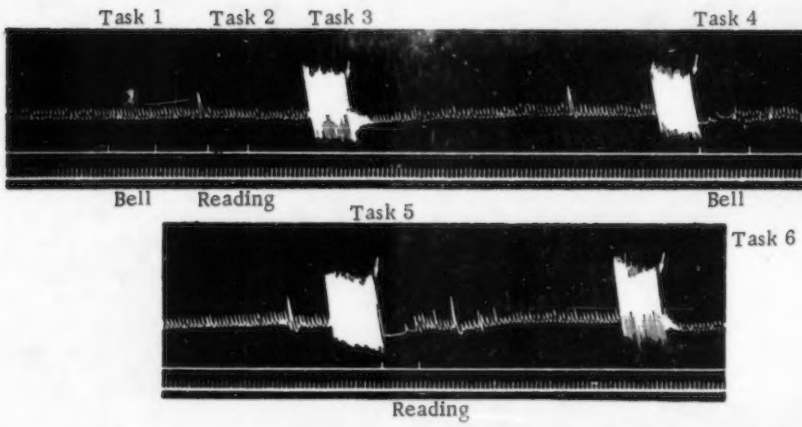

Fig. 3. Respiratory changes following hyperventilation (subject N.). Interpretation of curves is the same as in Fig. 1.

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THE USE OF CERTAIN HORMONES OF THE HYPOPHYSIS AND ADRENALS IN THE PREVENTION AND TREATMENT OF SHOCK DUE TO PROLONGED COMPRESSION OF SOFT TISSUES

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Hormone preparations are being increasingly used in surgical practice, especially in the management of shock [1-4, 8, 9, 10].

Nevertheless, the results of experimental and clinical trials of hypophyseal and adrenal hormones in shock, and the indications for their use have been insufficiently studied, and many points in this problem remain controversial. In the treatment of the sequelae of prolonged compression of soft tissues (the crush syndrome), so far as we know these preparations have not been tried.

The object of this research was to study the therapeutic value of the adrenocorticotropic hormone of the hypophysis (ACTH), cortisone, desoxycorticosterone acetate (DOCA) and noradrenalin in the treatment of shock arising during prolonged compression of the soft tissues.

METHOD

Experiments were carried out on white rats weighing 130-200 g. The soft tissues of both thighs were compressed by the application of special screw-clamps for eight hours. The volume of tissue compressed in each thigh was 5-6 cm^3.

The following items were studied: the behavior of the animals, the duration and rate of their survival, the body temperature and the number of eosinophils in the peripheral blood [5]. These indices were determined before, and 16-20, 48 and 72 hours after the application of the clamps. The results obtained were treated statistically.

The dosage of the hormones and the methods of their administration are mentioned in the description of the results.

RESULTS

The total number of experiments was 165, divided into 15 series (Table 1).

In the control experiments, the application of the clamps elicited a violent defensive reaction, which lasted for 1-2 hours. General depression then gradually developed and progressed. The body temperature fell to 35-30° 16-20 hours after application of the clamps, and in some experiments it fell even below 30°. The number of eosinophils was considerably reduced, and in most experiments they disappeared completely. After 48 hours the changes described had progressed in some animals, and in others a relative degree of normalization was observed (Table 2). Compression of the soft tissues caused necrosis of the skin and muscles of the limbs, as a result of which in the majority of experiments the limbs sloughed off 2-4 days after application of the clamps. From the results of the 50 control experiments, the rate of survival was 24.6% and the duration of survival 32 hours. The presence of marked variations in the resistance to trauma in different branches of rats made it necessary for us to carry out special control experiments in every case.

It is seen from the results in Table 1 that the preliminary intramuscular injection of ACTH for a period of seven days before infliction of trauma (series 2), in a dose of 3 units/kg twice daily, increased the animals' resistance. Six rats of this series actually survived more than 5 days, and the duration of survival of the rest was on the average 55 hours, against a mean survival period of the control animals of 17 hours. The difference between the mean results was statistically significant (probability of not arising by chance 0.970).

In these series of experiments (series 3, 4 and 5) the therapeutic value of ACTH was studied. The drug was injected in various doses (1, 3 and 10 units/kg) one and nine hours after removal of the clamps, and then twice a day thereafter. The rate and duration of survival of the animals receiving ACTH were higher than in the control animals. The best results were obtained when the preparation was given in a dose of 3 units/kg. In this series of experiments a comparatively high survival rate (60%) was observed, together with a relatively long survival period (48 hours). Compression of the limbs caused less severe disturbances than in the animals of the other experimental series. The body temperature after trauma (see Table 2) remained at a higher level; the number of eosinophils 72 hours after trauma had returned to the initial level in the majority of the animals which survived, thus demonstrating the normalization of the functions of the hypophysis—adrenal cortex system. It must be pointed out that large doses of ACTH (10 units/kg) led to a more pronounced hypothermia after the infliction of trauma, by comparison even with the

TABLE 1. Influence of ACTH, Hydrocortisone and DOCA on the Survival Rate and Duration After Infliction of Trauma

Series no.	Series of experiments	No. of animals: total	No. of animals: surviving	No. of animals: dying	Mean duration of survival (in hr)	Probability of significance of difference between mean survival period compared with control
	Trial of therapeutic and prophylactic action of ACTH					
1	Control	17	4	13	17± 4,9	
2	Prophylactic admin of ACTH (3 units/kg twice daily)	10	6	4	55± 6,9	0,970
3	Ther. admin. ACTH (1 unit/kg)	15	5	10	39± 7,0	0,987
4	Ther. admin. ACTH (3 units/kg)	15	9	6	43± 6,2	0,998
5	Ther. admin. ACTH(10units/kg)	15	6	9	55±10,9	0,999
	Trial of therapeutic action of hydrocortisone					
6	Control	10	—	10	36± 6,7	
7	Admin. of DOCA (30 mg/kg)	10	—	10	55± 9,3	0,875
	Trial of therapeutic action of DOCA					
8	Control	10	6	4	66±13,8	
9	Admin. of DOCA (2.5 mg/kg)	10	6	4	53±19,0	
10	Admin. of DOCA (5 mg/kg)	10	3	7	54±18,7	
11	Control	10	3	7	46±16,3	
22	Admin. of DOCA (5 mg/kg)	10	2	8	46±12,8	
	Trial of therapeutic action of noradrenalin					
13	Control	10	2	8	33± 6,0	
14	Noradrenalin (0.1 ml)	10	2	8	71± 8,3	0,998
15	The same (0.2 ml)	10	3	7	45±11,4	

control experiments, which was evidently due to the considerable decrease in the general reactivity of the animals resulting from the action of the drug.

Hydrocortisone was injected intramuscularly in a dose of 30 mg/kg one hour after removal of the clamps, again 8-10 hours later, and subsequently once daily. As a result of the injection of hydrocortisone the condition of the animals improved slightly. Whereas in the control animals the body temperature 20 hours after infliction of trauma had fallen on the average of 21.6°, the temperature of the animals receiving hydrocortisone remained at the level of 30.8°. (The difference is statistically significant.) The duration of survival of the animals receiving hydrocortisone was 55 hours, and that of the controls only 36 hours. Analysis of the results obtained shows that the use of hydrocortisone was less effective than the administration of ACTH for the same purpose. An obvious and characteristic action of the preparation was to decrease edema. Edema in the traumatized limbs of animals receiving cortisone was less marked than in the control animals.

The therapeutic efficacy of DOCA (a 0.5% oily solution) was studied in 50 experiments, 20 of which were controls. Desoxycorticosterone acetate was injected intramuscularly in a dose of 2.5 mg/kg (series 9) and 5 mg/kg (series 10 and 12), one hour after removal of the clamps, and then once on each successive day after infliction of the trauma. As a result of administration of the drug after trauma, a marked edema of the traumatized tissues developed, and secondary hemorrhages appeared much more frequently after removal of the clamps. In the small dose, the drug had no appreciable effect on the rate and duration of survival of the experimental animals, but in the larger dose (5 mg/kg), on the other hand, it had an adverse effect, lowering the survival rate of the animals after trauma (see Table 1). The body temperature of the animals receiving DOCA was 1.7° lower than in the corresponding control experiments 20 hours after trauma, and 2° lower 48 hours after trauma (Table 2). The eosinophils, as a rule, disappeared completely from the peripheral blood.

Noradrenalin, in a dose of 0.1 ml (1:20,000), had no effect on the survival rate of the animals, although the duration of their survival after infliction of the trauma was appreciably lengthened, on the average from 33 hours to 71

TABLE 2. Changes in the Body Temperature and the Number of Eosionphils in the Peripheral Blood after Infliction of Trauma

Series no.	Series of experiments	Initial findings		Findings after trauma					
				after 20 hrs		after 48 hrs		after 72 hrs	
		body temperature	no. of eosinophils	body temperature	no. of eosinophils	body temperature	no. of eosinophils	body temperature	no. of eosinophils
	Trial of therapeutic and prophylactic action of ACTH								
1	Control	36,8°	45	35,0°	0	33,1°	265	—	—
2	Prophylactic admin. of ACTH	37,9°	250	36,5°	0	37,4°	0	36,5°	320
3	Ther'tic admin. ACTH (1 unit/ kg)	37,8°	280	34,6°	134	31,6°	0	—	—
4	Ther' tic admin. ACTH (3 units/ kg)	37,0°	278	35,2°	22	34,8°	45	—	185
5	Ther'tic admin. ACTH (10 units/ kg)	37,2°	344	33,6°	—	32,8°	0	36,3°	34
	Trial of therapeutic action of hydrocortisone								
6	Control	37,5°	78	21,6°	0	—	—	--	—
7	Admin. of hydrocortisone (30 mg/ kg)	37,5°	58	30,8°	0	—	—	—	—
	Trial of therapeutic action of DOCA								
8	Control	36,7°	555	34,2°	10	37,3°	45	35,3°	—
9	Admin. of DOCA (2.5 mg/ kg)	37,4°	465	35,1°	22	36,4°	144	35,6°	—
10	Admin. of DOCA (2.5	36,9°	665	34,5°	0	36,7°	0	35,7°	22
11	mg/ kg)	37,0°	110	36,2°	0	36,0°	0	—	—
12	Control Admin. of DOCA (2.5 mg/ kg)	37,4°	665	34,5°	0	34,0°	0		
	Trial of therapeutic action of noradrenalin								
13	Control	37,0°	1 200	32,1°	12	—	—	—	—
14	Admin. of noradrenalin (0.1 ml)	37,1°	600	34,3°	12	36,4°	45	35,0°	33
15	Admin. of noradrenalin (0.1 ml)	37,7°	368	30,7°	33	36,6°	0	34,0°	156

hours (the difference between the mean values is significant). In a larger dose (0.2 ml) noradrenalin was less effective. The duration of survival of the experimental animals in this series of experiments was increased by only 12 hours over that of the control animals. Whereas small doses of noradrenalin improved the condition of the animals, large doses of the drug had the opposite effect. For instance, 20 hours after infliction of the trauma, the average body temperature of the animals in the control group was 32.8°, and after administration of noradrenalin in series no. 14 (0.1 ml) 34.3°, and in series no. 15 (0.2 ml) 30.7°. The difference between the mean values of the body temperature in the series in which noradrenalin was given in different doses was statistically significant (the probability of not arising by chance 0.984). Other conditions being equal, according to out observations the change in the body temperature in small animals after infliction of trauma fairly accurately reflects the degree of severity of the general disturbances.

The results of the work thus showed that ACTH and, to a lesser degree, hydrocortisone improve the animal's condition after infliction of severe trauma, as a result of which the rate and duration of survival of the traumatized animals are appreciably increased. The positive effect of these preparations is evidently due to their anti-inflammatory action and also to their influence on the permeability of the vascular walls, as a result of which the development of massive edema of the traumatized tissues is prevented.

In contrast to this, DOCA, which is a relative antagonist of the glucocorticoids, does not improve the condition of the traumatized animals but, on the contrary, in large doses it aggravates it.

Noradrenalin maintains the vascular tone and lengthens the survival period of the animals. These findings are in agreement with clinical observations showing the value of noradrenalin in shock [6, 7].

SUMMARY

Experiments were performed on 165 rats. The therapeutic efficacy of ACTH, hydrocortisone acetate and noradrenalin was tested in prolonged crush-syndrome. Special clamps were applied to the thigh for a period of 8 hours.

Prophylactic and therapeutic administration of ACTH improved the condition of the animals; as a result of this, survival rate and the life span were considerably increased. Cortisone also improved the state of the animals and lengthened their lives. Analogous results were obtained in noradrenalin administration. DOCA aggravated the animals' condition by increasing edema of the injured tissues and hemorrhages. In large doses DOCA exerted a negative effect on the survival and the life span of experimental animals.

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AN EXPERIMENTAL ANALYSIS OF THE PHENOMENON OF SPINAL SHOCK

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In connection with our findings that the sensory links of the somatic reflex arc are mainly affected in spinal shock, and also with É. A. Asratyan's [1] data on the consequences of section of the posterior half of the spinal cord, the experimental investigation of the degree of involvement of the various links of the vegetative (especially vasomotor) reflex arcs in spinal shock is of great interest.

Total transverse section of the spinal cord not only modifies the activity of the skeletal musculature, but it also alters the activity of the vegetative functions. In 1859, Claude Bernard [7] showed that, after high section of the spinal cord, the circulation of the blood is affected, primarily by a sharp fall in the arterial pressure. A similar phenomenon was also observed by Bezold [6] in 1863. He showed that the rise in arterial pressure which is normally observed during stimulation of various nerves is absent after total section of the spinal cord. Ludwig and Trie [8] also found a fall in the arterial pressure after total section of the spinal cord and showed that the level of the blood pressure may be again raised by electrical stimulation of the cross section of the caudal segment of the divided spinal cord.

A fall in the arterial pressure after high section of the spinal cord was also found in experiments by many other workers [5, 1, 2, 4, 9] and also in our own experiments, carried out on a large number of adult dogs. The increase in pressure in response to stimulation of the peripheral end of the divided spinal cord, previously observed [8], indicated that the function of the efferent vasoconstrictor neurone was essentially unaffected and that, consequently, the fall in the arterial pressure after high section of the spinal cord is due to changes in the functional state of other links of the reflex arc.

The object of the present investigation was to carry further the study of the problem whether depression of the sensory part of the reflex arc is the main cause of the disturbance of the reflex activity of the vasomotor centers of the spinal cord.

METHOD

In order to elucidate this problem which we had set ourselves, a series of experiments was carried out in which the different columns of the caudal segment of the divided spinal cord were stimulated in as localized a manner as possible, and the ensuing changes in the arterial pressure were examined. Observations were made on twelve adult dogs. Total section of the spinal cord was performed under morphine-ether-chloroform anesthesia in ten dogs at the level of the first thoracic segment, and in two dogs at the level of the fourth cervical segment. After section, the anesthesia was terminated and the remainder of the experiment was undertaken without anesthesia. Division of the spinal cord at the level of the fourth cervical segment, and the whole of the subsequent experiment, were carried out with the aid of artificial respiration.

The different columns of the spinal cord were stimulated by immersible electrodes, the ends of which were coated with special electrically insulating lacquer, so that only a very small part of the surface of the electrodes remained uncovered, in direct contact with a given area of the spinal cord. The columns of the caudal segment of the divided spinal cord were stimulated by means of an induction current from a Dubois-Reymond coil or by means of a stimulator. Stimulation lasted 20 seconds with intervals of 4 minutes between stimulations (after many tests we came to the conclusion that this was the optimal duration of stimulation). In two dogs—Belyanka and Bobik—besides stimulating the various columns of the spinal cord, we also stimulated the sciatic nerve by means of specially made immersible electrodes. To prevent transfer of loops of current to the cranial end of the divided spinal cord, the latter was earthed and additionally an area of cord 1-1.5 cm long was resected to increase the gap between the cranial and caudal ends of the divided spinal cord.

RESULTS

At the moment of division of the spinal cord the arterial pressure rose sharply and stayed at a high level for approximately 3-4 seconds, after which it gradually (for 50-80 seconds) fell. After total section of the spinal cord the arterial pressure fell on the average by more than 50% of its initial level. Its usual value was 40/50 mm Hg. When the arterial pressure fell still lower, which happened after division of the spinal cord at the level of the fourth cervical segment and also when hemorrhage was profuse during exposure of the spinal cord, we gave intravenous injections of a mixture of 10% calcium chloride with 40% glucose solution (into the femoral vein). This measure was highly effective in every case. In the dog Zheltushka, for

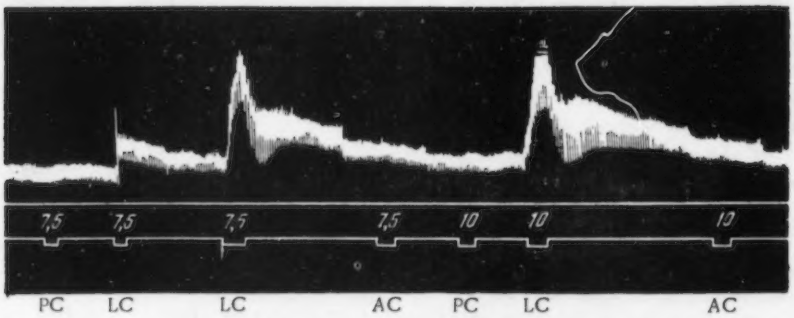

Fig. 1. Arterial pressure in the dog Bokser during stimulation of the anterior, posterior and lateral columns of the caudal end of the spinal cord, divided at the level of the first thoracic segment. Significance of the curves (from above down): arterial pressure; zero line; stimulation marker. The figures indicate the tension of the current (in volts), the letters—the area stimulated (L. C.—lateral columns, A. C.—anterior columns, P. C.—posterior columns). Intervals between stimulations 4 minutes.

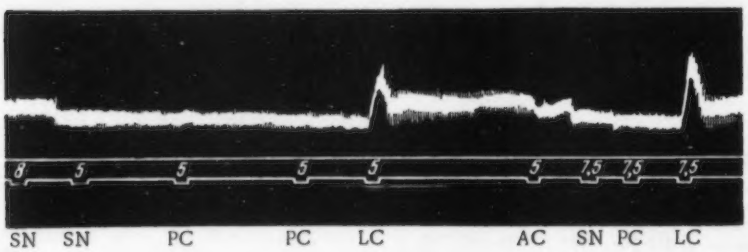

Fig. 2. Arterial pressure in the dog Belyanka during stimulation of the sciatic nerve and the anterior, posterior and lateral columns of the peripheral end of the divided spinal cord by means of an electric current. Legend as in Fig. 1. Intervals between stimulations 4 minutes. (SN—sciatic nerve).

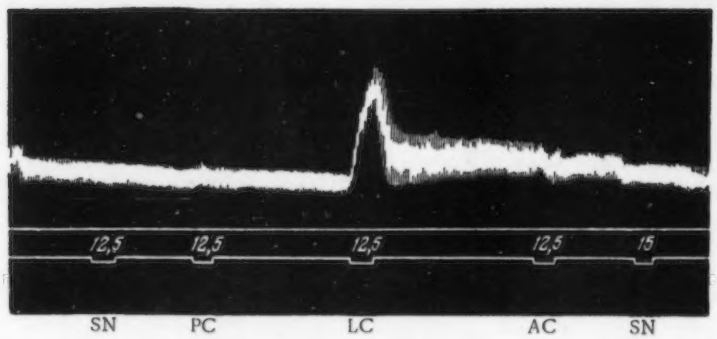

Fig. 3. Arterial pressure in the dog Bobik during stimulation of the sciatic nerve and of the anterior, posterior and lateral columns of the peripheral end of the divided spinal cord. Legend as in Fig. 1.

instance, as the result of profuse hemorrhage during exposure of the spinal canal the arterial pressure fell to 20/15 mm Hg even before division of the spinal cord, and it continued to fall. By means of two injections of calcium chloride with glucose (2 cm^3 of 10% calcium chloride and 8 cm^3 of 40% glucose on each occasion) we were able to raise the general level of arterial pressure to 80/60 mm.

The body temperature of the dogs fell to 34-36° after section of the spinal cord, and during acute experiments we therefore applied hot-water bottles to the dogs. After the animals had recovered from anesthesia and the arterial pressure had become stabilized at a definite level, stimulation was given to the different columns of the spinal cord.

Stimulation of the lateral columns of the spinal cord always elicited a quite strong and prolonged rise of the arterial pressure, which did not fall to its original value when the stimulation was discontinued. Stimulation of the anterior columns sometimes led to a slight increase in the arterial pressure, which rapidly fell to its initial level on discontinuing the stimulation, although occasionally the level of the arterial pressure remained unchanged. Stimulation of the posterior columns usually caused no change in the arterial pressure, and the changes if they did occur were extremely small and transient increases (Fig. 1).

In two dogs—Belyanka and Bobik—we stimulated the sciatic nerves in addition to the anterior, posterior and lateral columns of the caudal segment of the divided spinal cord. In the dog Belyanka the spinal cord was divided at the level of the fourth cervical segment. Division of the spinal cord and the subsequent experimental procedure were conducted with the aid of artificial respiration. In the dog Bobik the spinal cord was divided at the level of the first thoracic segment.

In all cases stimulation of the sciatic nerve caused no change in the arterial pressure either in the dog Belyanka or in the dog Bobik. Stimulation of the anterior and posterior columns also left the arterial pressure almost completely unchanged.

Stimulation of the lateral columns of the divided spinal cord caused a very considerable increase in the level of the arterial pressure in both dogs (Figs. 2 and 3).

In all our experiments in which the different columns of the divided spinal cord were stimulated in as localized a manner as possible, it was found that direct stimulation of the descending spinal tracts, connected with neurons situated in the lateral horns of the caudal end of the divided spinal cord, was always accompanied by a considerable rise in the level of the arterial pressure.

Stimulation of the sciatic nerve and of the posterior and anterior columns of the spinal cord, i.e., of pathways not connected with the above-named neurones, either caused no change whatsoever in the arterial pressure level, which was most often observed, or it led to a very slight increase in the pressure.

These facts may be explained by disturbances of the excitation level of the sensory links of the vasomotor reflex arc.

Our experimental results thus confirm the truth of the view developed by É. A. Asratyan, namely that in spinal shock it is mainly the sensory part of the reflex arc in which changes take place.

SUMMARY

Acute experiments were conducted on adult dogs with local stimulation of various tracts of divided spinal cord (caudal section). A considerable pressure rise always accompanied stimulation of the lateral columns of divided spinal cord. Stimulation of the sciatic nerve, posterior and anterior columns either had no effect whatsoever on the arterial blood pressure (which was observed more frequently) or its rise was insignificant. These facts may be explained by disturbed excitation of the sensory links in the vasomotor reflex arc.

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THE RENAL FUNCTION AFTER INJURY TO THE THALAMO-HYPOTHALAMIC REGION OF THE BRAIN

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There is an extensive literature on the question of the influence of the thalamus on the secretion of urine. In the regulation of the activity of the kidneys, great importance is attached to the grey matter of the brain situated in the tuber cinereum, the infundibulum, the corpora mammillaria and the region of the third ventricle. Experimental research has shown that injury to these divisions of the thalamus usually results in polyuria [1, 5, 9, 11, 13]. An increase in the secretion of urine may also be caused by puncture of the tuber cinereum and infundibulum in hypophysectomized animals [1, 7, 8].

It has been stated that the renal blood flow is under control of nerve cells situated in the tuber cinereum [2]. Strong evidence in favor of the participation of the thalamus in the regulation of the secretion of urine is given by the results of observations on patients with diabetes insipidus. In these cases pathological changes were found only in the hypothalamus; no organic changes were found in the hypophysis [4, 6, 10, 12, 13, 14].

In all these investigations, however, no consideration was paid to the renal mechanisms determining the character and degree of the changes in diuresis after disturbances of the subcortical nervous formations. The present work was devoted to the elucidation of this problem.

METHOD

Chronic experiments were carried out on dogs with ureteric fistulas, fashioned by I. P. Pavlov's method. We used two methods of causing injury to the brain: 1) mechanical destruction of part of the brain and 2) injection of alcohol into the brain substance. Hexobarbital was used as general anesthetic. The animals were bound in the prone position. The skin incision was usually made on

*Deceased.

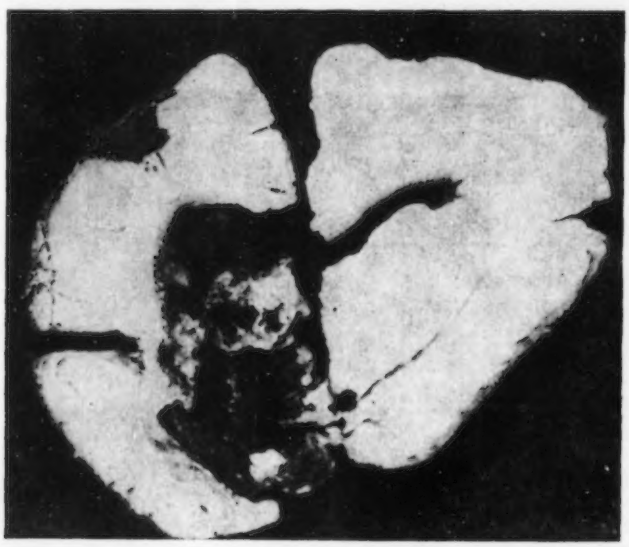

Fig. 1. Morphological picture of the left thalamo-hypothalamic region of the brain after mechanical injury.

the side of the skull where the brain was to be injured. After division of the skin and fascia, the temporalis muscle was mobilized with a raspatory, so far as possible without damaging it. A burr-hole was then made in the bone, 1-1.5 cm in diameter, at a previously calculated point. Hemorrhage was usually slight and easily stopped by one of the following measures: molten wax, hypertonic glucose solution and hemostatic gauze. When the bleeding was under control, the dura mater was opened. Mechanical injury to the brain was caused by means of a special device, contructed in É. A. Asratyan's laboratory, and the alcohol was injected by means of a syringe. The direction and depth of introduction of the cannula of the apparatus or of the needle of the syringe was determined in accordance with the size and shape of the dog's skull. The volume of alcohol injected was 0.5-0.7 ml. Finally, the wound was closed in layers. The experiments commenced not sooner than 2-10 days after the operation. The diuresis was studied after a known high water intake (60 ml of water/kg body weight of the dog), the glomerular filtration by means of inulin, the urea clearance, the renal blood flow and the maximum secretion by means of diodrast, the tubular reabsorption of water and urea and the plasma filtration fraction. A general analysis of the urine was also carried out.

RESULTS

Experiments were performed on seven dogs; in two of these animals the subcortical formations were destroyed mechanically, and in the remaining five by alcohol. This was because serious drawbacks appeared in the mechanical method of causing injury to the brain. The operation was very often fatal in its outcome. It was easy to damage other parts of the brain as well as the subcortical ganglia, and a large hematoma often developed (Fig. 1). The method of alcohol injection may to some extent limit the zone of injury and avoid further brain destruction and hematoma formation. By this method, moreover, the size of the zone of injury of the brain can be estimated to some extent, for it depends on the volume of alcohol injected, the rate of its injection and the pressure used.

Macroscopic examination showed that injection of alcohol caused injury to the nuclei of the optic thalamus and the subthalamic region, but in the cerebral cortex only small areas of damage were observed (Fig. 2).

After injury to the diencephalon, the animals for a few days displayed a pain reaction, low mobility, delay in taking food, disturbances of coordination of movement, disorders of the cardiovascular system, respiration and thermoregulation. These manifestations gradually became less pronounced and, finally, 10-15 days after operation, were no longer found.

The experiment showed that unilateral injury of the thalamo-hypothalamic region of the brain not only affects the animal's general condition, but also produces significant changes in renal activity (see Table). This was shown in the early period after operation by inhibition of the urine secreting function of the kidneys. In 5 of the 7 experimental animals in the first 5-10 days after injury to the diencephalon, a fall in diuresis was observed, amounting to 30.77-42.86%. A tendency not only toward an oliguric type of water diuresis, but also to a delayed and extended type was detected under these circumstances. Whereas in normal conditions the maximum urine secretion took place in the first two hours after intake of water, after injury to the diencephalon the diuretic process was drawn out, and the maximum diuresis was observed only after 2-3 hours or even later.

In individual animals, along with a fall in the diuresis there was a fall in the glomerular filtration, which, however, did not exceed 3.11-4.14%. Such a very slight fall in the glomerular filtration cannot, of course, be the essential cause of a comparatively large-scale fall in diuresis. In certain cases, moreover, the decrease in urine secretion was associated with an increase in the rate of glomerular filtration.

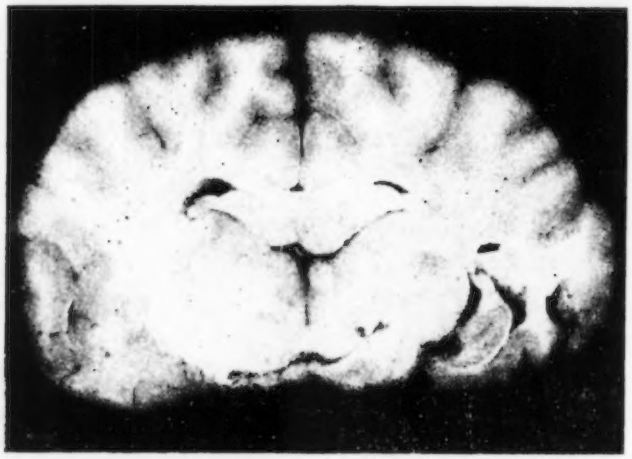

Fig. 2. Changes in the left thalamo-hypothalamic region of the brain after injury by injection of alcohol.

TABLE. Figures Relating to the Renal Function After Injury to the Left Thalamo-Hypothalamic Region of the Brain Due to the Injection of 0.3 ml of Alcohol (the dog Chita, Weight 10 kg)

Indices of the functional state of the kidneys	Before injury to the diencephalon (mean figures from 3 experiments)	After injury to the diencephalon		
		Day after operation		
		12th	16th	27th
Diuresis (in ml/min)	3,9±0,20	2,70	3,10	4,40
Plasma urea concentration (in mg%)	28,90	22,90	40,80	16,58
Glomerular filtration (inulin) (ml/min)	28,30±7,97	30,80	25,90	31,50
Urea clearance (in ml/min)	27,30	57,40	31,82	29,40
Renal blood flow (plasma flow) (diodrast) (in ml/min)	124,98±8,14	135,51	156,10	166,70
Reabsorption of water in tubules (in %)	77,44±3,39	88,60	86,10	73,33
Ratio between coefficient of urea clearance and coefficient of inulin clearance	0,72	1,86	1,22	0,93
Maximum tubular secretion, in mg iodine (per min)	17,56±2,87	21,08	25,80	31,00
Volume of plasma cleared of diodrast by a unit of functioning tubules (in ml/min)	7,38±1,68	6,50	6,10	5,30

According to our findings, the phenomenon of oliguria mainly developed in consequence of an increase in the reabsorption of water in the tubular portion of the nephron.

Side by side with decrease in glomerular filtration the presence of a disturbance of the intrarenal hemodynamics may be assumed. Immediately after injury to the diencephalon, the picture of disturbed renal circulation was apparent in the fall in the coefficient of diodrast clearance, which indicated a reduction in the blood flow through the kidney, and also in the increase in the fraction of the plasma filtered in the glomeruli. In several cases the fall in the renal blood flow was calculated to be 33.01-36.15% and the increase in the plasma filtration fraction reached 153.98-163.04%. This suggests that the fall in the blood supply to the renal parenchyma was greatest in the tubular portion of the nephron, and was due to constriction of the efferent arterioles of the glomeruli. It is very probable that the increase in the tone of the glomerular vessels and also the increase in the intensity of reabsorption of water in the tubules were associated with the production of adrenalin and of other vasoconstrictor substances. Meanwhile the decrease in the power of the tubules to secrete diodrast cannot be attributed purely to the disturbance of the renal circulation. This can be concluded from the fact that a fall in the maximum secretion to 13.94% was observed at the same time in the individual experiments.

It must be mentioned that injury to the thalamo-hypothalamic region of the brain did not always lead to the same type of result. Depending on the depth of the brain injury, either transient or more lasting disturbances of the renal function could be observed. The most marked abnormalities were observed after mechanical injury to the subcortical area of the brain. At subsequent periods of observation, a definite tendency toward polyuria was observed. In some experiments the secretion of urine exceeded the normal value by 14.64-25.26%. In the overwhelming majority of cases the increase in diuresis was accompanied by an increase in the glomerular filtration to 67.71-127.85%, of the urea clearance to 58.19-110.26%, of the renal blood flow to 24.90-33.58% and of the maximum secretion to 46.92-60.48%. Later the renal function was gradually restored, and two or three weeks after operation all the indices of the work of the kidneys were approximately at their original value.

The findings described thus afford further confirmation of the reports in the literature that the central regulation of the activity of the kidneys is effected by means of the diencephalon, and they demonstrated the reversibility of all the abnormalities of renal function arising after injury to the thalamo-hypothalamic region of the brain. This is evidence of the great powers of compensation possessed by the the nervous system in the regulation of the vegetative functions of the body. The role of the subcortical nerve centers is not confined to the regulation of the renal circulation. It seems that, independently of this activity, they also influence the specific functions of the tubules.

SUMMARY

The functional condition of the kidneys was studied in unilateral injury of the thalamo-hypopthalamic region of the brain in 7 dogs with Pavlov ureteric fistulae. At first, injury to the diencephalon provokes a fall in diuresis, glomerular filtration, urea clearance, renal blood flow and maximal secretion. Then on the 5th-10th postoperative day, a rise of the renal function indices is seen. The changes in the renal activity are reversible and functional in character. The role of the diencephalon is not limited to the control of the renal blood supply. Independently of this fact it influences the specific function of the tubules.

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CORTICAL COORDINATION IN DOGS WITH EXPERIMENTAL ATHEROSCLEROSIS

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The object of the present research was to investigate the cortical activity in dogs with experimental atherosclerosis, the plan being to study the influence of the defensive reaction [2, 3, 4, 7, 8, 16, 17 and others], developing in the course of artherosclerosis, on other reactions with a different biological significance.

METHOD

Chronic experiments were performed on five dogs. Atherosclerosis was produced in three of these animals by feeding with cholesterol and methyl thiouracil [15]. The remaining dogs acted as controls, and received methyl thiouracil alone in the same dose as the first animals (1.5g). In some dogs a conditioned postural reaction was first developed, permitting recordings to be made of the ballistocardiogram and other hemodynamic indices in the course of a chronic experiment, both in normal conditions and during the development of atherosclerosis [19]. After its consolidation, this postural reaction was also used for recording [4] the electroencephalogram (EEG). The conditioned postural reaction obeys the general laws of cortical activity [10, 11, 12]. We therefore took it into account when analyzing the influence of atherosclerosis on conditioned reflex activity. In other dogs, conditioned defensive reflexes, produced by the Staritsin-Petropavlovskii technique, were preliminarily reinforced. Control investigations on intact dogs gave rise to an EEG of identical character, in spite of intervals of many days in the experiments in which it was recorded. This can be explained by the fact that, during these intervals, the conditioned postural reaction common to all the experiments was systematically reproduced in other experiments to study the hemodynamics [20]. The same thing was also observed in other dogs which, during the interval in the recording of the EEG, were trained in the technique of the conditioned defensive reflexes.

RESULTS

The conditioned defensive reactions in intact dogs are shown in Figs. 1a and 1b. In the first second of ringing the bell, the dog raised its paw and held it in a state of continuous contraction during the whole time of action of the conditioned stimulus. The respiration showed absence of deep inspirations and expirations, and also of dyspnea. The EEG before administration of cholesterol and methyl thiouracil is shown in Figs. 2a and b. Relatively frequent oscillations of considerable amplitude arose in all leads of the EEG, i.e., the phenomenon of synchronization of the rapid waves was observed. During the application of the signal and the development of the conditioned defensive reaction, synchronization of the rapid waves was also observed in the EEG.

Feeding the dogs with 6-methyl thiouracil and cholesterol was then begun, and continued for $4\frac{1}{2}$ months. The development of atherosclerosis of increasing severity in the dogs was shown by biochemical and morphological investigations and also by hemodynamic changes. No similar changes were found in control dogs which received 6-methyl thiouracil alone. The developing artherosclerosis caused persistent changes in the EEG after 2-$2\frac{1}{2}$ months (Fig. 2b, c, d). The onset of atherosclerosis led to a fall in the voltage of the EEG waves in the experiments on all the dogs (Fig. 2b). In this period of the experiments, the EEG in the premotor and sensomotor area of one hemisphere was appreciably different from that from other areas of the brain. For instance, waves appeared in the premotor area with a frequency of 30-40 cps and a positive phase, with a relatively higher amplitude than the waves in the other leads (Fig. 2c). The waves in the sensomotor area were less frequent and of high amplitude (Fig. 2d). In all the control dogs a fall in the voltage of the EEG appeared on the 11th-13th day of feeding with methyl thiouracil, but on the 16th-17th day this disappeared altogether, and the EEG was not only restored to normal, but even showed an increase in the degree of synchronization (Fig. 2f, g).

The fall in the voltage of the EEG waves reached its maximum degree five months after the beginning of feeding with the atherosclerosis-producing substances. For instance, 28 days after the end of feeding with these substances, when the blood cholesterol concentration was on

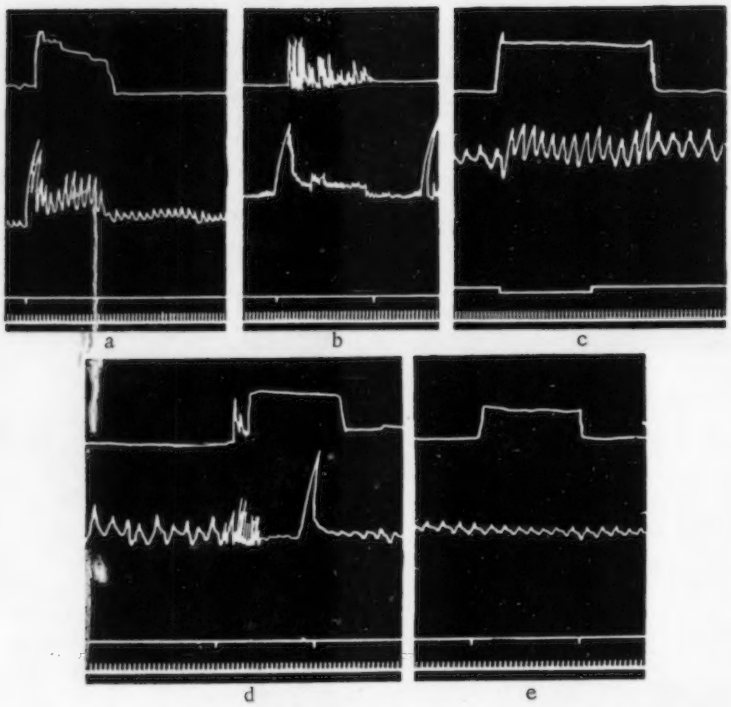

Fig. 1. The effect of methyl thiouracil and cholesterol on the conditioned defensive reflex. a) conditioned defense reflex before feeding with methyl thiouracil and cholesterol; b) disturbance of the conditioned defensive reflex due to feeding on methyl thiouracil and cholesterol (sixth month after production of experimental atherosclerosis); c) conditioned defensive reflex before feeding with methyl thiouracil alone; d) disturbance of the conditioned defensive reflex on the 11th-13th day of feeding with methyl thiouracil; e) recovery of the conditioned defense reflex in spite of feeding with methyl thiouracil (30th day of feeding with methyl thiouracil). Significance of the curves (from above down): contraction of the muscles of the dog's left hind limb; respiration; marker of the conditioned stimulus; time marker (1 second).

the average 2.5 times higher than its initial level, waves of very low amplitude were observed in the EEG (Fig. 3a). It might be thought that the absence of high-amplitude waves was due to the diminished activity of the cortical cells. In order to elucidate the physiological mechanism of the persistent fall in the voltage of the EEG waves, we gave chlorpromazine. This drug is known to block the tonic influence of the reticular system on the cortical cells [1, 23]. If the fall in voltage was due to a diminution in the activity of the cortical cells, then after the administration of chlorpromazine, the voltage of the EEG waves would either remain unchanged or would fall still further. The opposite changes were found, however, and the results were the same in all the experimental dogs. Administration of chlorpromazine (2.5 mg/kg) led to an increase in the voltage and to the restoration of synchronization in the EEG in all leads (Fig. 3b). In contrast to the original EEG, however, the waves observed were slower and of a relatively high amplitude.

After the administration of chlorpromazine, the dogs lay in another position with considerable relaxation of their skeletal musculature. The experimenter's call, i.e., the signal for adoption of the previous postural reaction as was usual during the experiment, again caused a fall in the voltage of the EEG waves (Fig. 3c). In other words, during excitation, which was the cause of the new pose adopted by the dog after the administration of chlorpromazine, with a corresponding unconditioned stimulus, another stimulus was applied which was of special significance for the animal. The latter stimulus evoked excitation, but in another reflex arc, corresponding to the specially developed postural reaction.

The suggestion that 6-methyl thiouracil, by blocking the activity of the thyroid gland, depresses the activating

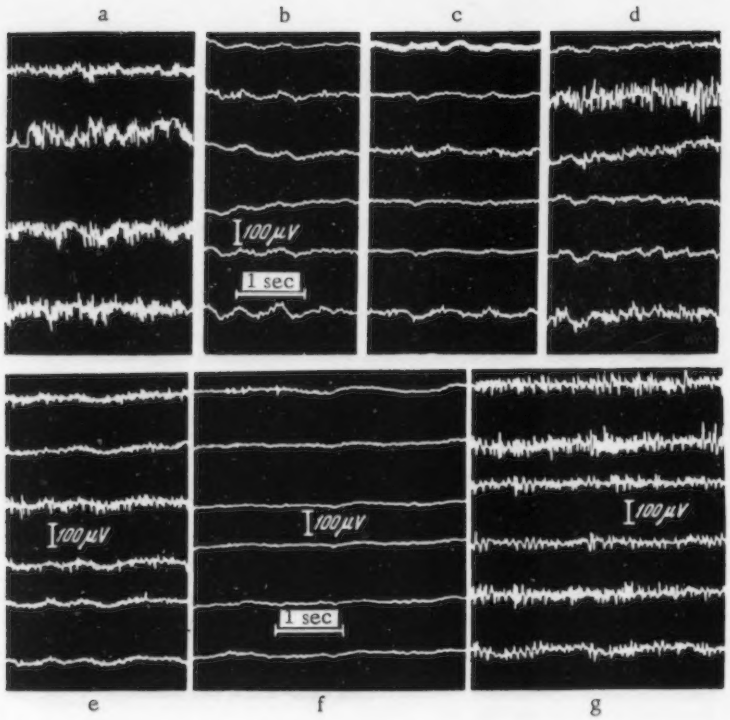

Fig. 2. Changes in the EEG in dogs after feeding with methyl thiouracil and cholesterol. a) before feeding with methyl thiouracil and cholesterol; b, c, d) fall in the EEG voltage after feeding with methyl thiouracil and cholesterol (2-2½ months from the beginning of production of experimental atherosclerosis); e) before feeding with methyl thiouracil; f) fall in the EEG voltage on the 11th-13th day of feeding with methyl thiouracil alone; g) recovery and increase in the EEG voltage in spite of continuing feeding with methyl thiouracil (30th day of feeding with methyl thiouracil). Significance of the curves (from above down): EEG from the premotor, sensomotor, parietal and occipital areas of the right cerebral hemisphere (Fig. 2a); EEG from the premotor, sensomotor, parietal and occipital areas of the right hemisphere; EEG from the premotor and sensomotor areas of the left hemisphere (Fig. 2b, c, d, e, f, g).

influence of the reticular system and thereby brings about a fall in the voltage of the EEG, was also wide of the mark. Evidence against it was provided by the results of the administration of 6-methyl thiouracil alone to control dogs and of the experiment in which chlorpromazine was given to dogs with experimental atherosclerosis.

The facts described thus show that the fall in the voltage of the EEG in atherosclerosis was not due to a diminution of the activity of the cortical cells. On the contrary, the fall in the voltage of the EEG waves was caused by their desynchronization, reflecting the presence in the cerebral cortex of foci of excitation, evidently differing in their rhythm and stimulating reactions of differing significance to the animal.

At that period of the experiments when persistent desynchronization was seen in the EEG, the reactions to stimulation of different biological significance (food stimuli, showing a dog of the opposite sex, or a cat) showed predominance of a general state of inhibition. During the experiment to record the EEG the dogs tried to replace the postural reaction that had been developed with a pose involving less strain on the somatic musculature. The conditioned defensive reactions were also considerably modified. It is clear from Fig. 1b that, at the signal, the dog had lost its ability to produce a tonic form of contraction of the limb. Throughout the whole time of action of the conditioned stimulus, the motor reaction bore the character of a rhythmic withdrawal of the paw. When the paw was raised, the dog did not lower it, although the signal continued to act. In dogs receiving 6-methyl thiouracil, the disturbances were ill-defined and brief in duration. They were observed for 3-5 days (on the 11th-13th days of feeding with 6-methyl thiouracil), and did not subsequently arise (Fig. 1d and e).

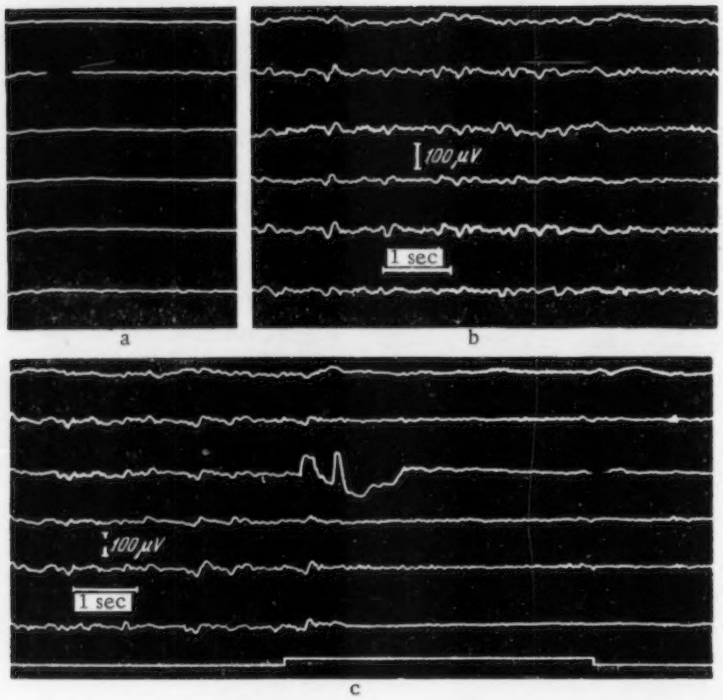

Fig. 3. The EEG in a dog with atherosclerosis after administration of chlorpromazine. a) EEG before administration of chlorpromazine; b) EEG after administration of chlorpromazine; c) EEG during the action of the sound stimulus in association with chlorpromazine. Legend as in Fig. 2. Last line at the bottom of Fig. 3) stimulus marker.

In these experiments the character of the respiratory component began to change: the respiration was superficial, dyspnea developed and isolated deep inspirations and expirations were frequently observed.

After necropsy, performed six months after the beginning of feeding with 6-methyl thiouracil and cholesterol, atheromatous lesions of the vessels, especially the vessels of the base of the brain and of the heart, were found in all the dogs. No signs of atherosclerosis were found in the dogs receiving 6-methyl thiouracil alone.

The concrete analysis of the effect of experimental atherosclerosis on the cortical activity requires a basic assumption in respect of the general nature of the pathology of the animal as a whole. We have in mind the physiological nature of protective inhibition as a defensive reaction of the body.

Since the work of I. P. Pavlov [8] on this problem, many new findings have accumulated. It has been shown, for instance, that in extraordinary situations, the hypothalamus and vegetative nervous system take part in the response reaction of the animal. The participation of these divisions of the nervous system in the reaction of the animal to excessively strong stimuli, which has been detected qualitatively, has been called "Reilly's stimulation reflex" [22, 24, 26, 28, 29]. Moreover, the hypophysis and adrenal glands are also involved in this reaction [27]. It may be postulated from these facts that protective inhibition, as a defensive reaction arising in response to a stimulus causing disturbance or pathological change in the animal body, is a special unconditioned reflex, i.e. this is a reaction based on the process of excitation. It may be thought that the defensive reflex bears special relationships toward its stimuli, the central organization and the effector component. Pavlov [8] mentioned the possibility of such a reflex in 1909, calling it the "self-righting reflex."*

The interconnections between the cerebral cortex and the hypothalamus, and also data on the conditioned reflex reproduction of the defensive reflex [5, 6, 7, 8, 9] testify to the participation of the cortical level and to the possibility of the formation of this reflex as a signal reaction. Like other unconditioned reactions, however, the defensive reflex has it cortical representation, with the participation of which the cerebral cortex forms the outward behavior in accordance with its biological significance.

* Pavlov considered that the phenomena of protective inhibition and the phenomena of recovery were interconnected but not identical. He declared, for instance: "We have said that they are somehow connected, that inhibition is caused by disturbance, but we have never said that inhibition and recovery are identical". ["Pavlov's Wednesday", Izd. AN SSSR 3, 311 (1949).]

We thus hold the view that when the defensive reflex is dominant, the animal's outward behavior is concerned with an active reaction. The latter usually takes the form of that postural reaction in which the minimum number of muscle components is implicated in an active state. When the defensive reflex, like any other reflex, becomes dominant [18], of necessity it is accompanied by the coordinated inhibition of other reactions, for example those based on alimentary and sexual excitation, so that the vegetative control of all the formations associated with somatic activity is reduced to the minimum. This leads, from our point of view, to a state of "general inhibition" of the animal, which is especially pronounced in the period of maximum intensity of the pathological process and of mobilization of the defensive reflex. This state is not the result of inhibition of the entire cerebral cortex, but is the external expression of the presence of a dominant excitation, associated with the defensive reaction, and of an associated inhibition of all other reactions functionally incompatible with the first.

In our experiments the development of atherosclerosis was accompanied by the appearance of desynchronization in the EEG. Other authors, engaged on the study of this disease, have also noted a fall in the voltage of the EEG [13, 21]. It must be emphasized that the phenomenon of desynchronization arises in nonspecific form in various species and in different pathological conditions, and also during conditioned reflex activity in the healthy animal. We consider that the views regarding the phenomenon of protective inhibition that were expressed above enable the general electroencephalographic phenomena (desynchronization and synchronization) to be related to the pattern of formation of cortical coordination, and help to elucidate the nature of the disturbance of this coordination during the development of a pathological process.

Investigations of different cases of cortical activity of the healthy animal have shown that the formation of cortical coordination passes through two stages—a stage of conflict and a stage of increasingly complex coordination [10, 12]. Signs of the stage of conflict are ambiguity in behavior, desynchronization in the EEG and the tense character of the vegetative components, and signs of the stage of increasingly complex coordination are a uniform pattern of behavior, synchronization in the EEG and normalization of the vegetative components [12].

From our point of view, the attempt to extend coordination patterns studied in the healthy animal to the cortical activity in atherosclerosis is perfectly justified. In fact, when the sick dog is placed on the apparatus, antagonistic interaction must inevitably arise in the animal between qualitatively incompatible reactions, based on different forms of unconditioned excitation. On the one hand, excitation associated with the dominant defensive reaction is responsible for the outward behavior of the animal in accordance with the demands of this reaction,—the dog actively attempts to adopt a pose with the minimum number and effort of the muscular components, during which the vegetative control of the somatic activity is considerably diminished, and is mobilized mainly for the needs of the defensive reaction. On the other hand, however, a qualitatively different form of excitation arises in response to the environmental stimulus or the defensive signal. As a result of the previous training, the environmental stimuli stimulate the postural reaction which was developed before the onset of the pathological process, quite different in the composition and intensity of its muscular components. The same thing takes place also during the action of the conditioned stimulus causing the reaction of withdrawing the limb.

As a result of the simultaneous action of stimuli associated with qualitatively different reactions, an antagonistic interrelationship develops between the latter, i.e. conditions are created for the formation of cortical coordination. The unstable character of the hitherto stable conditioned reaction, the desychronization in the EEG and the tense character of the vegetative components, as in the healthy animal, are the expressions of the stage of conflict in coordination, i.e. of the super-threshold excitation of the dominant reaction and of the high level of super-threshold excitation of the competing potential reaction. In the formation of external behavior during atherosclerosis, the defensive reaction may occupy the role of either a dominant or a competing potential reaction with a high level of super-threshold excitation. In both cases, however, the immediate mechanism of coordination is the same.

The distinguishing feature of the cortical coordination in atherosclerosis, by comparison with that in the healthy animal, is the drawn-out character of the stage of conflict.

Our experiments show that dogs with atherosclerosis lose the ability to maintain a high level of excitation throughout the whole time of action of a conditioned stimulus. Coordination is formed as a result of mobilization of the excitation in the reflex arc of the dominant reflex [18]. It may therefore be considered that in the case of atherosclerosis the cause of the drawn-out character of the stage of conflict is a disturbance of brain-stem mechanism [25] ensuring optimal tone of the central excitation necessary for normal coordination. This is possible, for in atherosclerosis, especially in the cerebral form, which is well marked in dogs, the blood supply of the brain is disturbed.

SUMMARY

The phenomenon of the EEG voltage is caused not by the reduced activity of the cortical cells, but by the presence of excitations which are uniform in rhythm, as well as of stimulating reactions possessing different significance for the animal. One of these excitations is connected with the protective reaction arising from atherosclerosis, while another is associated with the reaction mobilizing the organism in another direction, which does not conform to the requirements of the protective reaction. The formation of cortical coordination takes place during the simultaneous action of the stimuli provoking both of these reactions. In

these conditions of desynchronization, the EEG components reflect the conflict stage in the formation of cortical coordination. The effect of experimental atherosclerosis on the cortical activity includes reduction of the cortical excitation as a result of which the conflict stage becomes protracted and is not transformed into the final stage—that of formed coordination—for a long period of time.

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†See C.B. translation.

THE INFLUENCE OF RADIAL ACCELERATION ON THE SECRETION OF THE INTESTINAL GLANDS IN DOGS

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In previous communications [4, 5] we studied the influence of radial acceleration on the secretion of the salivary and gastric glands. Some difference was found in the duration of the course of the response reaction under these conditions. Whereas unconditioned reflex salivation usually returned to approximately the original value 3-10 minutes after the action of the acceleration, in the case of the gastric glands this period was prolonged to several hours. The cause of this phenomenon was evidently to be found in differences in the regulation of the functions of the organs themselves.

It was thought to be of some interest to study the secretion of the glands of the small intestine, in the regulation of which an important part is played by local nervous mechanisms [1, 2, 3, 6, 7, 8].

METHOD

Experiments were carried out on four dogs, with loops of intestine fashioned by Thiry's method. In three of these dogs (Seryi, Belaya and Nochka), portions of intestine 25 cm long were resected from the upper division of the small intestine, and in one (Volchikha), from the lower division at a distance of 30-40 cm from the cecum. Intestinal secretion was evoked by the application of a mechanical stimulus (a rubber tube 12 cm long). In addition, in some experiments, observations were made on the periodic secretion of intestinal juice. The experiments lasted 3-6 hours. Besides measuring the volume of secretion, the proportion of solid and liquid portions of the intestinal juice was investigated, and the enzymic activity was determined – amylase, by Wohlgemuth's [9] method, and phosphatase, by the method of L. S. Fomina, S. Ya. Mikhlin and G. K. Shlygin [7].

During the investigations the animals were kept on a constant mixed diet, and before the experiment were starved for 18 hours. Radial acceleration was applied by rotating the animals on a centrifuge of radius 3.6 m. We studied the effect of acceleration in a cranio-caudal direction, of magnitude from 5 to 9 g, and lasting from 20 to 60 seconds. As a preliminary measure, before the experiments to study the effect of radial acceleration, the normal secretion of the intestinal glands was established for a period of $1\frac{1}{2}$-2 months. Altogether 192 experiments were carried out on 4 dogs.

RESULTS

Immediately after the application of radial acceleration of a magnitude of 5 g and lasting 20 seconds, no special changes could be observed. However, 24 hours after rotation a more than threefold increase in the secretion was observed, by comparison with the initial figures (9.3 ml; Fig. 1).

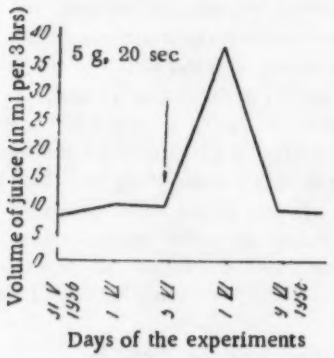

Fig. 1. Change in the secretion of intestinal juice in response to a mechanical stimulus in the dog Volchika under the influence of radial acceleration.

It may be seen from Fig. 1 that the secretion of intestinal juice rose to 38.3 ml 24 hours after the action of the acceleration. On the fourth day it was observed to have returned to its original level. During the period of the effect of acceleration, and also after 2-4 days, the volume of solid residue in the juice was increased.

After the action of radial acceleration of magnitude 7 g for one minute, more prolonged changes were observed in the secretion. Figures obtained in the experiments on the dog Belaya are given in the table.

It can be seen from the table that, on the day of the experiment in which acceleration was applied, a slight decrease in the intestinal secretion was observed. Subsequently the secretion gradually increased and, in the experiment on December 26, i.e., 15 days after rotation, it

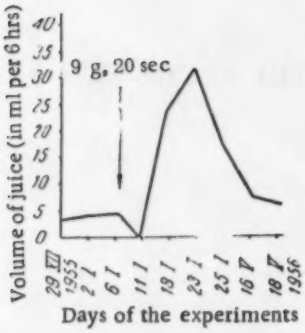

Fig. 2. Change in the periodic secretion of intestinal juice in the dog Nochka under the influence of radial acceleration.

reached 68.6 ml (by comparison with the original values of 21.7-29.6 ml). At the same time the proportion of solid part of the juice increased, and there was a rise in the activity of the enzymes, especially amylase. The activity of the enzyme phosphatase, which rose on the fourth day after rotation, then fell sharply, and in the subsequent experiments no phosphatase was found. We obtained similar results, with the action of acceleration of the same magnitude, in the dog Seryi also. On the 17th day, the secretion had fallen to its original level.

An acceleration of 9 g lasting 20 seconds was followed by much more sharply defined changes. These changes were observed not only during investigation of the intestinal secretion in response to the mechanical stimulus, but also in the state of periodic secretion. As an illustration we show certain findings obtained in the dog Nochka (Fig. 2).

It may be seen from Fig. 2 that the periodic secretion of juice in this dog over a period of 6 hours amounted to 3.2-4.0 ml. No changes were present during the day after the action of acceleration. After 5 days, however, total disappearance of periodic secretion was observed. On subsequent days the periodic secretion rose sharply. In these experiments the duration of the individual periods was observed to be increased. In several cases the secretion became nearly continuous for an interval of 6 hours. The aftereffect was prolonged.

The effect of radial acceleration is thus to cause considerable changes in the secretory activity of the intestinal glands. After the repeated action of acceleration smaller changes were usually observed, although an increase in the acceleration again led to obvious changes in the secretory activity. As an example, in Fig. 3 are shown the results of experiments carried out on the dog Seryi.

It may be seen that the intestinal secretion after the repeated action of acceleration of 7 g lasting 1 minute was close to the original value. After the application of an acceleration of 8 g for 1 minute, however, an increase in secretion was again observed.

The results described above show that, under the influence of radial acceleration, considerable changes take place in the secretory glands of the intestine in dogs. A very characteristic feature is the duration of the changes observed. Whereas, according to our findings, the after effect on the gastric glands lasts a few hours, that on the secretory activity of the intestinal glands is measured in days, and with high values of acceleration, in weeks.

It must be pointed out that in response to the action of other factors, for example anoxia, on animals a prolonged after effect on the intestinal glands is also observed [6,8]. The cause of such a prolonged after effect evidently lies in the peculiarities of the local nervous reflex regulation of the functions of the small intestine.

While these changes were taking place in the intestinal secretion, the general condition of the dogs remained perfectly satisfactory.

SUMMARY

The author studied the effect of positive radial acceleration on the secretion of intestinal juice in dogs. The

TABLE. Influence of Radial Acceleration on the Secretion of Intestinal Juice in the Dog Belaya (Experiment of 1956)

Index	Experimental conditions								
	Background		7 g, 1 min.	Aftereffect					
	Dec. 6	Dec. 8	Dec. 11	Dec. 13	Dec. 15	Dec. 18	Dec. 26	Dec. 28	Dec. 30
Volume of juice secreted in 3 hours in response to a mechanical stimulus (in ml)	21,7	29,6	17,0	21,3	38,1	42,4	68,6	27,4	27,2
Amylase activity	8	8	4	8	32	8	32	16	32
Phosphatase activity	450	200	450	300	750	50	0	0	0
Ratio of solid to liquid parts of the intestinal juice	1:5	1:7	1:5	—	1:2	—	1:5	1:2	1:2

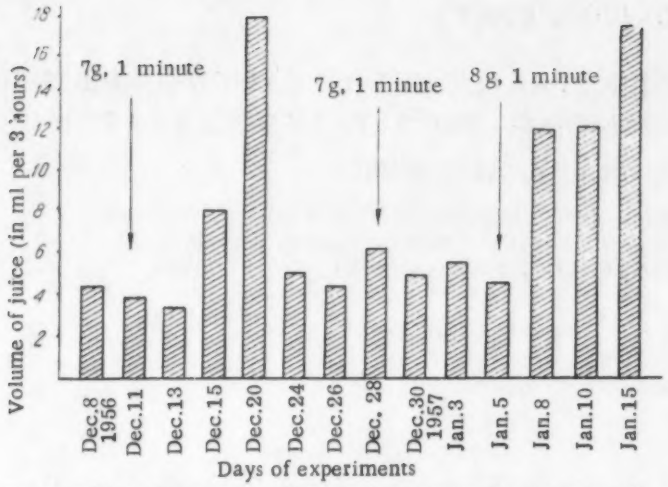

Fig. 3. Effect of repeated radial acceleration on the secretion of intestinal juice produced by application of the mechanical stimulus (the dog Seryi).

first exposure to acceleration was followed by a prolonged after-effect (from several days to several weeks). There was an increase of intestinal secretion, the activity of amylase and phosphatase became changed and a rise in the amount of the solid portion of intestinal juice was noted. In repeated exposure to the action of radial acceleration adaptational phenomena were noted in intestinal secretion.

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THE EFFECTS OF PHOSPHATE ADMINISTRATION ON PHOSPHORYLCREATINE, GLYCOGEN, AND LACTATE LEVELS IN ANIMAL MUSCLE

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(Presented by Active Member of the USSR Academy of Medical Sciences, S. V. Anichkov)

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Although a large body of published work can be found [2, 3, 7, 8] on the problem of the increase in the working capacity of animals following the administration of phosphate, little actual biochemical interpretation of the phenomenon has been offered. While some workers [2, 3] attributed the effect of phosphate to the stimulation of the central nervous system, Embden and co-worker [7] believed the main mechanism of its effect to consist in the stimulation of glucose phosphorylation in muscle directly. In view of the currently accepted importance of the high-energy phosphate compounds in muscular activity, it was felt desirable to establish the effect of phosphate administration on the levels of these compounds in muscle.

The present work deals mainly with the effect of administered phosphate on the muscle level of phosphorylcreatine—one of the most important sources of contractile energy in muscle, in the form of high energy phosphate which can be passed on to ATP. The intensity of glycolysis was also investigated simultaneously by following lactate and glycogen levels.

METHOD

White rats, weighing 130-170 g, were given 30 mg P as orthophosphate per kg intraperitoneally in the form of a 3.8% solution of NaH_2PO_4. Three groups of animals were used, and the phosphate administration was carried out under amytal anesthesia, under anesthesia combined with a nervous block (tying up of n. ischiaticus) or under denervation alone, respectively. The animals were killed by decapitation at various intervals after phosphate administration, the muscle (gastrocnemius) was immediately excised and frozen in liquid air, and portions of the frozen muscle were used for the estimation of phosphorylcreatin (according to A. M. Alekseeva), glycogen (by a micromodification of the Pflüger method) and of lactate (according to Barker and Sommerson).

RESULTS

There were no significant changes in the levels of the compounds under investigation in the muscles of control animals which were given normal saline solution instead of orthophosphate. In control animals the levels of phosphorylcreatine (PC) in muscle were 213-233 mg%, of glycogen—481-533 mg% and of lactic acid—37.3-53 mg%. Administration of phosphate had a specific effect on the levels of all three compounds, the effect being dependent on the time interval between the treatment and assay. Thus, 30 min after phosphate administration (Fig. 1), PC levels fell by 30%. It should be remembered that this drop in PC levels could not have occurred as a result of excessive mobility, since throughout the period the animals were in a state of relative rest and their behavior did not differ from that of the control rats. Within one hour of phosphate administration PC levels returned to normal, but continued rising, exceeding control levels by 58% at two hours. Thereafter the PC content fell gradually, but even six hours from the onset of the experiment, its levels in the muscles of treated animals were 36% above control values. This difference disappeared within 24 hours. The changes in glycogen levels after phosphate administration were less pronounced. Statistical analysis of the data showed a lack of significant effect in most of the time intervals investigated. The only exception was the two-hour interval, where a significant increase in glycogen levels of 18% (Fig. 2) was detected. In

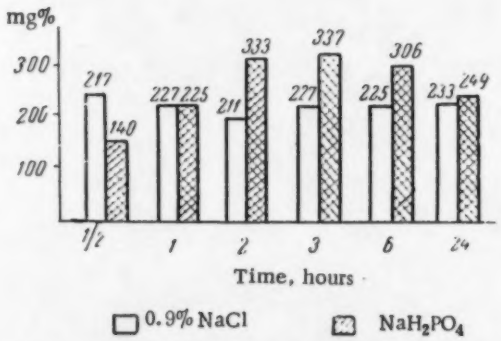

Fig. 1. Phosphorylcreatine levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

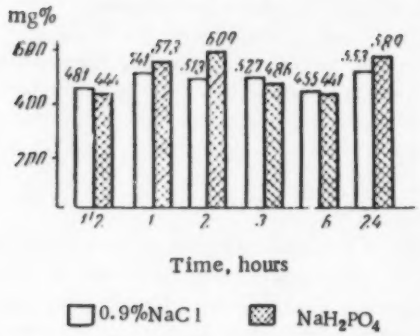

Fig. 2. Glycogen levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

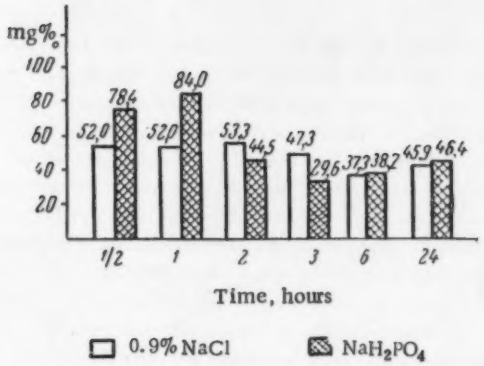

Fig. 3. Lactic acid levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

this connection it is interesting to note that Yakovlev [4] found that the change of glycogen content in starved cats given orthophosphate followed a similar time-sequence. Lactic acid assays showed that, after the administration of phosphate, lactate levels rose in the first hour, exceeding control levels by 58-61%, and then fell, returning to normal within two hours (Fig. 3).

Consideration of the over-all result suggests that during the initial period (30 min–1 hour) after phosphate administration the intensity of glycolysis increased, giving rise to the accumulation of lactate in the muscles. This was accompanied by a fall in PC levels. There was no significant simultaneous fall in glycogen levels. It would appear that the increase in muscle lactate had taken place at the expense of the blood glucose, rather than of the muscle glycogen. The possibility of this alternative was demonstrated by Yakovlev [4], who reported a fall in blood glucose levels after phosphate administration. It is characteristic that the changes observed in the present work took place in the absence of any increase in the mobility of the animals. Of greatest interest is the observation that, two hours after phosphate administration, there was a sharp increase in PC content in muscle, and some increase in glycogen levels, both changes favoring the increased potential for subsequent work, which may be the explanation for the increased working capacity following phosphate administration. The present results so far left unanswered the questions: What is the mechanism of phosphate effect on muscle chemistry and, was the total effect of phosphate a direct one on the muscle chemistry, or an indirect one on the central nervous system and mediated through its trophic effect on muscle?

In order to resolve this problem the experiments were repeated using animals in which the action of the higher nervous centers was excluded through the application of amytal anesthesia, or peripheral denervation, or both. In all these experiments the two-hour interval, which previously afforded the greatest degree of change, was employed. The biochemical changes observed were the same as described above, irrespective of whether the experiment was carried out under amytal anaesthesia, or with denervation of the limb, or with a combination of the two (table); phosphorylcreatine levels increased by 50-55%, while glycogen and lactate levels remained at approximately normal values. It was thus demonstrated that PC levels in muscle increased after phosphate administration without the mediation of the higher nervous centers, in anesthetized animals and where the nerve supply to the muscle

TABLE. Phosphorylcreatine, Glycogen and Lactate Levels in the Muscles of Animals, Two Hours After Phosphate Administration (in mg%).

Treatment	PC	Glycogen	Lactic acid
Normal saline	211±24,4	513±42,0	53,3±7,3
Orthophosphate (30 mg P/kg)	333±17,8	609±17,6	44,5±3,9
As above, under amytal anesthesia	328±17,6	602±23,1	49,0±8,0
As above, with anesthesia and denervation	322±15,4	543±17,0	41,2±3,2
As above, with denervation only	360±14,6	556±28,1	46,8±6,7

was disconnected. The results indicated that the effect of phosphate on muscle metabolism is a direct one on the tissue. It is, of course, impossible to exclude any adaptive-trophic effect of the vegetative nervous system but apparently in the present case the effect of the latter was of secondary importance. This assumption is also supported by the work of Veselkina [1], who noted the participation of the somatic nerves in the transmission of the trophic influence of the central nervous system on muscle PC levels. Also, Yakovlev [5] observed, in experiments with isolated muscle, distinct changes in the carbohydrate-phosphorus metabolism of the tissue when the phosphate concentration in the surrounding medium was raised.

It is feasible that one of the factors which limit the relatively low level of PC in muscle is the deficiency of inorganic phosphate. This is suggested by the fact that in muscle only 30-40% of the total creatine present is actually phosphorylated. The increase of the concentration of inorganic phosphate in muscle apparently affects the carbohydrate-phosphorus metabolism of this tissue. It is well known that in muscle the synthesis of high-energy phosphates can be accomplished either by a phosphorylation coupled to oxidation, or during glycolysis. Muscle PC can also be resynthesized by either a transphosphorylation with ATP, or with disphosphoglycerate [6]. The existence of such diverse pathways in muscle makes it difficult to decide unequivocally the nature of phosphate effect observed in the present experiments. One explanation which cannot be excluded is the intensification of oxidation phosphorylation with the subsequent PC synthesis by transphosphorylation from ATP to creatine. Further work is necessary for the fuller elucidation of the phenomenon.

In conclusion, it seems reasonable to advance the suggestion that the main mechanism in the increase of working capacity of muscle by phosphate administration consists in the raising of the levels of energy-rich phosphates in the tissue.

SUMMARY

Within two hours of the intraperitoneal administration of inorganic phosphate (30 mg/kg) to rats, phosphorylcreatine and glycogen levels in muscles increased markedly. Administration of general anaesthesia or the denervation of the lower extremity failed to abolish this effect.

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THE EFFECT OF GANGLIOBLOCKING AGENTS ON AFTER-DISCHARGES

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(Presented by V. V. Zakusov, Active Member of the AMN SSSR)

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The investigations of V. V. Zakusov and co-workers have established that substances which inhibit synaptic conduction in the central nervous system reduce the force and shorten the duration of after-discharges connected with precedent stimulation of the afferent and pyramidal tracts [3, 4].

These data and the fact that sequential processes play an important part in the activity of the whole nervous system prompted us to investigate the effect of ganglioblocking substances on after-discharge in the sympathetic ganglia. In the literature available to us, we could find no investigations especially concerned with this question.

After-discharges in the sympathetic ganglia are most apparent after brief (1-2 seconds) stimulation of the preganglionic fibers by stimuli 50-130 per second in frequency. One can observe a particularly pronounced and prolonged after-effect with the preliminary administration of physostigmine [8, 9].

The mechanism of development of after-discharges in the sympathetic ganglia is a particularly interesting question. After-discharge in a ganglion cannot be explained by time dispersion, because the general route between the stimulating and receiving electrodes is the same for all the fibers, and the difference in the rates of conduction and synaptic lag is not great enough to explain a persistent after-effect. D. Bronk and M. Larrabee [7] observed after-discharges when action potentials were led off from single postganglionic fibers of the stellate ganglion. On this ground, one can assume that after-discharges are connected with changes developing in the bodies of the ganglionic neurons or in the presynaptic endings. In the autonomic ganglia, after-discharge is evidently caused by continuing stimulation of the ganglionic cells due to liberation of chemical substances (acetylcholine, potassium ions) persisting after tetanus has ceased [6, 8-10]. The increased frequency and duration of after-discharges following the use of eserine, or in the case of a surplus of potassium ions or a deficiency of calcium ions, or with acetylcholine perfusions of the ganglion gives this theory considerable support.

METHOD

The experiments were performed on the stellate ganglion of cats anesthetized with chloralose (80 mg/kg intravenously) and urethan (0.5-1 g per animal intravenously). The thoracic cavity was opened from the 1st to the 6th-7th rib, under conditions of artificial respiration. The preganglionic trunk was transected at the level of the 5th-6th thoracic sympathetic ganglia, and stimulating electrodes were placed between the 2nd and 3rd or the 3rd and 4th rami communicantes. The bio-electric currents were led off from the inferior cardiac nerve, transected 3-4 cm from the ganglion. All the other postganglionic fibers and the 1st-6th ramus communicans were transected. The blood supply of the ganglion was left intact.

The preganglionic trunk was stimulated with supramaximal square-wave stimuli given at a rate of 100 per second to induce tetanus lasting one second. The duration of each stimulus was 0.1-0.5 millisecond.

The experiments were performed on a background of physostigmine (2-5 mg/kg) in order to intensify and prolong the after-effect. The ganglioblocking substances tested were tetraethylammonium (TEA), Hexonium (hexamethonium), Pentamine (methyliminodiethylene bis ethyldimethyl ammonium bromide) and Mekamine. All the substances were introduced into the femoral vein. The ganglioblocking agents were used in doses of 0.5-2 mg/kg.

RESULTS

The experiments showed a marked decrease in the after-effect after the administration of TEA in a dose of 0.5 mg/kg. This was particularly apparent after the use of the substance in a dose of 1-2 mg/kg. Figure 1, 1, for example, shows that tetanic stimulation of the preganglionic sympathetic trunk following the intravenous injection of physostigmine (3 mg/kg) was attended by a prolonged after-effect. The size of the individual discharges reached 50-60 μv. In a dose of 1 mg/kg, TEA (see Fig. 1, 2) almost completely prevented the development of the after-effect. At the same time, adequate conduction of nerve

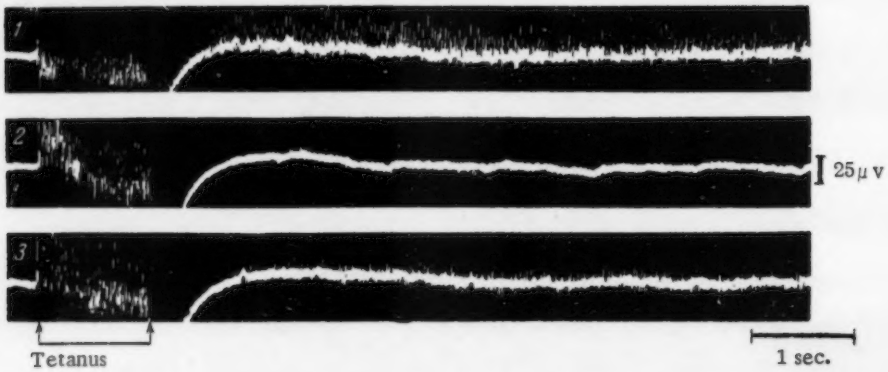

Fig. 1. Effect of tetraethylammonium on after-discharge in the stellate ganglion. 1) before administration of substance; 2) one minute after TEA injection (1 mg/kg); 3) 15 minutes after injection. Tetanus (beginning and end indicated by arrows); maximal square-wave stimuli lasting 0.5 millisecond each and given at a rate of 100 stimuli per second. Tetanic stimulation of the preganglionic fibers accomplished in one second. Experiment carried out on a background of the action of physostigmine (3 mg/kg).

impulses was retained in the ganglion. This was indicated by the pronounced potentials of the postganglionic fibers which were observed during the tetanus. Under the given experimental conditions, it was difficult to record their true value, because the experiments required maximal amplification of the action potentials. This was necessary in order to study the after-discharges, which usually ranged from 10 to 30 μv in value. Naturally, the potentials of the postganglionic fibers, which reached amplitudes of 500-700 μv (with electric stimulation of the preganglionic trunk), often exceeded the limits of the screen.

Fig. 1, 3 shows a tendency of the after-effect to reappear. After the use of TEA, due to the brevity of its effect, the complete restoration of the value and duration of the after-discharge could be easily observed.

Analogous results were obtained with Hexonium, Pentamine and Mekamine. In a dose of 1-2 mg/kg, all these substances prevented the development of after-discharges. The effect lasted longer than that produced by the TEA injection. The most lasting effect was induced by Mekamine. With regard to duration of effect, the ability of the experimental ganglioblocking agents to inhibit the after-discharge was parallel to their inhibitory influence on the interneuronal conduction of nerve impulses.

Fig. 2 shows the results of one of the experiments with Mekamine. The experiment was performed on a background of physostigmine (3 mg/kg intravenously). The top oscillogram (see Fig. 2, 1) shows brief tetanus (100 stimuli per second for 1 second) attended by a marked after-effect. Mekamine was given in two injections of 1 mg/kg each. After the first injection of the substance (see Fig. 2, 2), we noted a sharp decrease in the duration and amplitude of the after-discharges, which were observed only during the first $2\frac{1}{2}$-3 seconds after tetanus. The second injection of 1 mg/kg Mekamine (see Fig. 2, 3) caused the after-discharges to decrease still further in voltage; no after-discharges were observed after the first $1\frac{1}{2}$ seconds following tetanus.

As well as the ganglioblocking agents, we tested substances from other pharmacological groups (Barbamyl and Novocain). Both these compounds are known to inhibit the autonomic ganglia quite actively. The results of these experiments demonstrated that the effect of Barbamyl or Novocain on the after-discharge is similar to that of the ganglioblocking substances. We observed the decrease or complete inhibition of the after-discharges after the administration of 20-30 mg/kg Novocain or 15-20 mg/kg Barbamyl. This effect developed considerably earlier than did the block of ganglionic transmission in this case.

The results of the experiments indicate that the experimental ganglioblocking agents reduce or completely prevent the development of after-discharges. This effect was usually attended by a decrease in the amplitude of the bio-electric currents in the tetanic series. Complete block of ganglionic transmission did not occur in this case. Therefore, the ganglioblocking substances primarily prevent the development of the after-effect, but higher doses are required before they can block the conduction of nervous excitation in the ganglion with electric stimulation of the preganglionic fibers.

The action mechanism of the experimental substances seems to be based on their ability to inhibit the synaptic apparatuses of the ganglionic neurons, at the same time increasing the latter's threshold of sensitivity to acetylcholine. However, one should take into account the fact that certain substances (Novocain) can decrease the production of acetylcholine. It is interesting that the parameters characterizing the function of the neurons and of their syn-

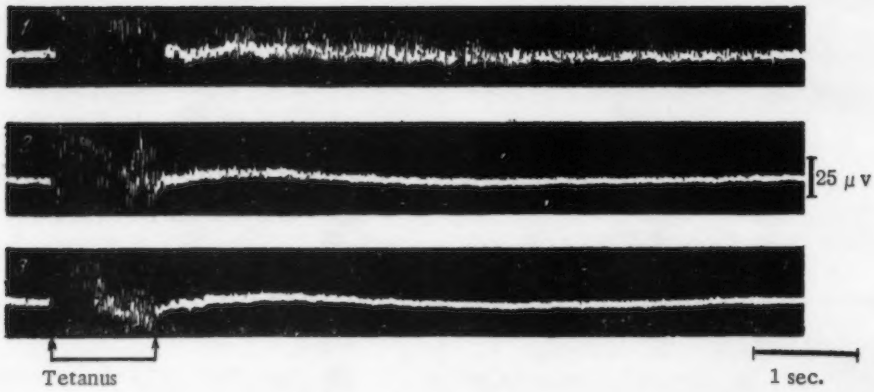

Fig. 2. Effect of Mekamine on after-discharge in the stellate ganglion. 1) before administration of substance; 2) five minutes after Mekamine injection (1 mg/kg); 3) five minutes after second Mekamine injection (1 mg/kg). Other symbols the same as in Fig. 1.

aptic formations change in about the same way under the influence of ganglioblocking agents. Thus, for example, we have shown that ganglioblocking substances, as well as reducing the excitability of the ganglia, decrease their functional mobility, increase the time of interneuronal conduction and, as established in this investigation, diminish and abbreviate the after-discharge.

This brings up the question of how these functional characteristics would change under the influence of other substances capable of inhibiting ganglionic transmission by various mechanisms of action. We used Barbamyl and Novocain for purposes of comparison. It was found that both substances caused the same changes in the excitability, lability, latent period and after-discharges of the sympathetic ganglia as did the experimental ganglioblocking agents.

On the basis of these data, one can assume that other substances which inhibit synaptic conduction in the autonomic ganglia ought to induce analogous effects. However, in evaluating the common principles of action of ganglioblocking agents, one should remember that the changes which lead to disturbance of ganglionic transmission may be brought about by very different mechanisms. This also applies to the anatomical localization of the effect of ganglioblocking agents and to the influence of these substances on the biochemical processes important to the transmission of nervous excitation. For example, Z. Zaimis and co-workers [5] believe Mekamine and Hexonium to have different mechanisms of action. These authors associate Mekamine's blocking effect with its intracellular action; Hexonium, on the other hand, affects the synaptic formations of the ganglionic neurons.

N. V. Vysotskaya [1, 2] established in our laboratory essential differences as to the type of effect exerted by ganglioblocking agents on certain aspects of metabolism. Investigation of the metabolism of the phosphorus groups in the superior cervical ganglion showed that nicotine, pachycarpine, TEA and Pentamine reduce the content of adenosine triphosphoric acid (ATP) in the ganglion; Hexonium is not active in this respect. The fact that ATP restores conduction of stimulation in the ganglion if the conduction has been inhibited by nicotine, pachycarpine, TEA or Pentamine indicates that the change in the ATP content plays an important part in the action mechanism of most of the experimental ganglioblocking agents. The blocking effect of Hexonium does not change under the influence of ATP. One must therefore suppose that the mechanism of Hexonium's blocking effect differs from that of Pentamine and TEA. According to the classification of W. Paton, W. Perry and co-workers, however, these substances all belong to the competitively acting groups of compounds [11, 12].

Therefore, with the aid of the physiological parameters characterizing the interneuronal transmission of nervous stimulation, we were able to obtain an idea of the general outlines of the action of ganglioblocking agents. To gain a more intimate understanding of the mechanism of their depriming effect, the biochemical or physicochemical changes of the synaptic formations and of the nerve cells as a whole should evidently be investigated.

SUMMARY

The author studied the effect of tetraethylammonium, Hexonium, Pentamine, Mekamine, Barbamyl and Novocain on the after-discharges in the cat's stellate ganglion. As demonstrated by the experimental results, all these substances reduce or completely inhibit the development of after-discharges. This action is accompanied by a decrease in the amplitude of the action potentials in the tetanic series during electric stimulation of the preganglionic fibers; but complete block of the ganglionic transmission does not occur.

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THE EFFECT OF PENTAPHENE AND STRYCHNINE ON THE POLYSYNAPTIC REFLEXES OF DIFFERENT SECTIONS OF THE SPINAL CORD

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(Presented by V. V. Parin, Active Member of the AMN SSSR)

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It has been established in a series of works [5, 15, 16] that the polysnaptic reflex arcs of the lumbar section of the spinal cord are more sensitive to the action of certain anesthetics than are the analogous reflexes of the cervical portion.

The purpose of this investigation was to ascertain whether other pharmacological agents (non-anesthetic) have a preferential effect on the caudal segments of the spinal cord.

The agents selected for this purpose were strychnine, an anesthetic antagonist, and Pentaphene (diethylaminoethyl ester of phenylcyclopentacarbonic acid), a cholinolytic substance which, like anesthetics, inhibits cerebrospinal activity.

METHOD

The experiments were performed on adult cats, the spinal cord of which had been sectioned at the level C_1 under deep anesthesia. The anesthesia was discontinued immediately after the transection, and the animal was kept warm and under artificial respiration for the duration of the experiment. We recorded the bioelectric currents of the biceps femoris and the biceps brachii developing in response to single threshold stimulations consisting of square-wave stimuli 0.12 millisecond in duration applied to the central sections of the transected sensory and mixed nerves of the extremities.

Pentaphene and strychnine were introduced intravenously. Preliminary experiments were carried out in order to establish the doses with which the clearest results could be obtained. This dose was found to be 10 mg/kg for Pentaphene and 0.05 mg/kg for strychnine. In each experiment, we injected the specified dose of the substance 2-3 times. Since the effects of the first and second injections were about the same, the results of all the observations were processed together.

The bioelectric potentials were recorded before the injection of the experimental substance and then 1, 3, 5, 10, 15 and 20 minutes after the injection. The value of the reflex response to a single stimulation was determined by measuring the area of the bioelectric currents. All the values (the areas of the bioelectric potentials) were calculated in percent of the original effect. The data obtained were statistically processed. A result with $d < 0.05$ was assumed to be authentic.

We performed a total of 15 experiments with Pentaphene on six animals and 16 experiments with strychnine on seven animals.

RESULTS

Experiments with Pentaphene. We observed inhibition of all the experimental spinal polysynaptic reflexes after the administration of Pentaphene, but the effect was more pronounced at the level of the lumbar reflex arcs. Fig. 1 (a and b) graphically depicts the changes in the average values of the reflex responses of the arm and leg muscles after the administration of Pentaphene. One can see a sharp deterioration of reflex activity the very first minute. The effect reached its maximal point the third minute. Thereafter, the reflex responses gradually increased. The character of the reaction was about the same regardless of whether the sensory nerves (a) or the mixed nerves (b) were stimulated. Fig. 2 gives data from one of the experiments. The figure shows the complete inhibition of the reflex responses of the biceps femoris muscle (c and d) after the administration of Pentaphene and the reduced, but evident response of the biceps brachii muscle (a and b).

Experiments with Strychnine. Although the administration of strychnine sharply intensified all the experimental polysynaptic reflexes, there was a much greater increase in the reflex responses of the biceps femoris muscle than in those of the analogous arm muscle. This is clearly shown in Fig. 1 (c and d). The reflex response of the biceps femoris muscle became 4-7 times stronger after the

* Deceased.

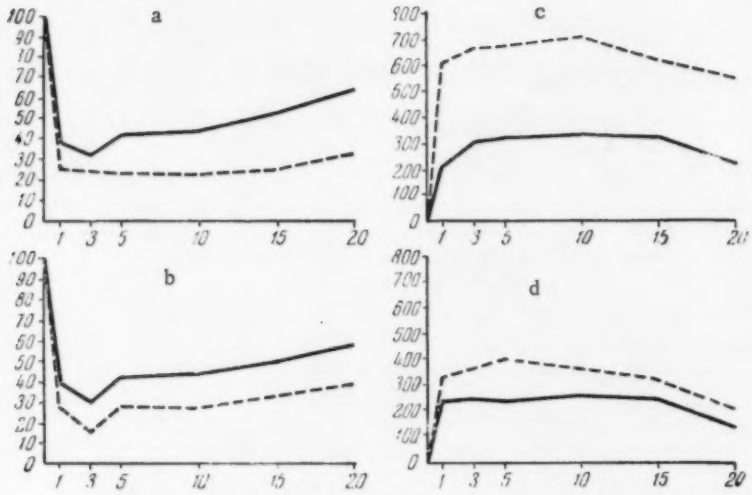

Fig. 1. Graph showing the effect of Pentaphene (a and b) and strychnine (c and d) on the polysynaptic reflexes of spinal animals. On axis of abscissa is shown the time (in minutes); on axis of ordinate is shown the area of the bioelectric reflex responses of the muscles (in percent of original value). a, c) reflex responses of the biceps brachii (——) and the biceps femoris (– – – –) muscles to stimulation of the sensory nerves (respectively, the medial cutaneous and saphenous nerves); b, d) reflex responses of the same muscles to stimulation of the mixed nerves (respectively, the ulnar and tibial nerves).

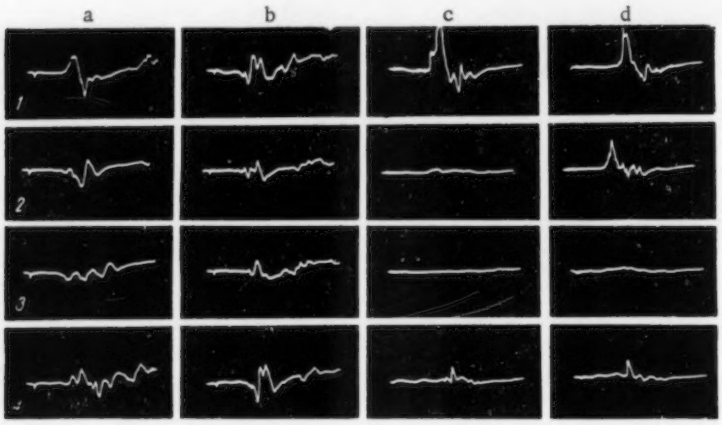

Fig. 2. Effect of Pentaphene on the polysynaptic reflexes of a spinal animal. a) biceps brachii muscle with stimulation of medial cutaneous nerves; b) biceps brachii muscle with stimulation of ulnar nerve; c) biceps femoris muscle with stimulation of saphenous nerve; d) biceps femoris muscle with stimulation of tibial nerve; 1) initial reflex responses; 2-4) respectively, 1, 5, and 10 minutes after the administration of 10 mg/kg Pentaphene.

administration of strychnine, while the response of the biceps brachii muscle became 2-3 times stronger. In this case, there was a greater degree of increase in the reflex response to stimulation of the sensory nerves.

With the administration of strychnine, as in the case of Pentaphene, the difference in value of the changes in the reflex reactions under comparison was statistically justified throughout the investigation.

Fig. 3 shows that strychnine causes greater intensification of the reflex responses of the biceps femoris muscle (c and d) than of the reactions of the analogous arm muscle (a and b).

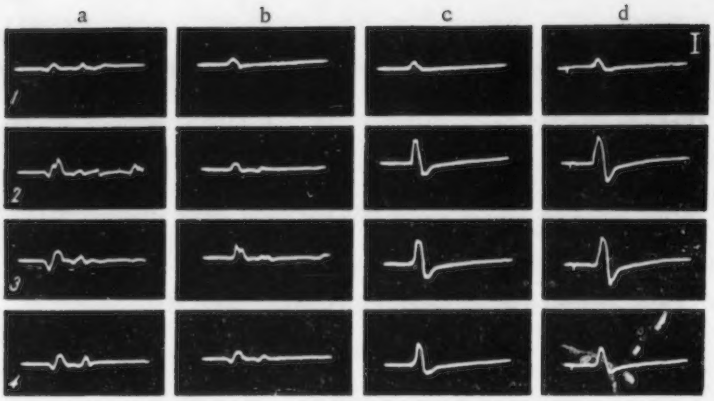

Fig. 3. Effect of strychnine on the polysynaptic reflexes of a spinal animal. a) biceps brachii muscle with stimulation of medial cutaneous nerve; b) biceps brachii muscle with stimulation of ulnar nerve; c) biceps femoris muscle with stimulation of saphenous nerve; d) biceps femoris muscle with stimulation of tibial nerve; 1) initial reflex responses; 2-4) respectively, 1, 5, and 20 minutes after the administration of 0.05 mg/kg strychnine. Calibration equals 1 mv.

From the literary data, it is known that in many pathologic conditions attended by injury to the motor apparatus, change in the cerebrospinal function moves in cranial direction.† When anesthetics are administered to intact animals, for example, the first changes observed in the animals are ataxia and paresis of the posterior extremities [7, 11-14]. A study of pathologic reflexes from the abdominal cavity of animals demonstrated that, first, asthenia of the posterior extremities develops, then paralysis of the latter and only after this does paralysis of the anterior extremities develop [9]. One of the first symptoms of rabies is mild paresis of the posterior extremities. In rabies, the posterior portion of the body becomes paralyzed before the anterior [8, 10]. In certain forms of tetanus, when the focus of injury is not predominant in the disease picture, the posterior extremities are affected before the anterior [3]. Paralysis in poliomyelitis usually travels from bottom to top [4]. The functions of the posterior extremities are the first to fail in high partial oxygen pressure [1, 2]. With the administration of Novocain and many anesthetics, the reflex activity of the lumbar portion of the spinal cord is more disturbed than that of the cervical region [5, 6, 15, 16].

These data suggest that the facts we obtained regarding the preponderant effect of Pentaphene and strychnine on the reflex activity of the caudal segments of the spinal cord are a specific case of a more general rule governing change in the reflex activity of the spinal cord under pathologic conditions. It is difficult to say at this time just what the reasons for and the mechanisms of this rule are.

SUMMARY

The effect of Pentaphene and strychnine on the polysynaptic reflexes of the cervical and lumbar sections of the spinal cord was studied on spinal cats by electrophysiological methods. The material was statistically analyzed. It was established that Pentaphene preponderantly depresses, and that strychnine enhances the reflex activity of the lumbar spinal cord segments.

It is known that it is toward the cranium that the changes of the spinal cord functions develop in many pathologic processes attended by affection of the motor apparatus. This suggests that the data obtained regarding Pentaphene and strychnine are a particular case of a more general rule governing the reflex action change occurring in the spinal cord in pathologic conditions.

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POTENTIALIZATION OF HYPNOTICS AND ANESTHETICS BY CENTRAL CHOLINOLYTICS

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(Presented by S. V. Anichkov, Active Member of the AMN SSSR)
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Investigations carried out by foreign and Russian scientists [1, 2, 5-13] have shown that many of the new cholinolytic agents generally designated as atropine simulants possess an ability to block the cholinergic structures of the central, as well as the peripheral, nervous system; in fact, the cholinolytic effect of some of these preparations is predominantly central. S. V. Anichkov has proposed that such substances (Difacyl, Diazyl, Pentaphene, Tropacine, Methyldiazyl, Aprophene, etc.) be specifically classified as central cholinolytics.

As we [3, 4] and the authors mentioned above have shown, in certain doses, central cholinolytics have a pronounced inhibitory effect on the brain, disturbing higher nervous activity, altering bioelectric activity and preventing arecoline and nicotine convulsions. In [5], S. S. Krylov, having studied the effects of Difacyl and Diazyl on the higher nervous activity of dogs, suggested the possibility of using these substances in combination with hypnotics.

We believe the study of how central cholinolytics influence the effects of hypnotic and anesthetic substances, with a view toward determining the possibility of their combined use to be of definite theoretical and practical interest.

METHOD

Experiments were performed on 1,000 white mice (males weighing 18-20 g) and 20 rabbits. The hypnotic and anesthetic substances used were Barbamyl, chloral hydrate, Hexenal and thiopental.

The central cholinolytics tested were: Difacyl (diphenylacetic acid β-diethylaminoethyl ester hydrochloride), Methyldifacyl (diphenylacetic acid β-diethylaminoisopropyl ester hydrochloride), Diazyl (benzilic acid β-diethylaminoethyl ester hydrochloride), Methyldiazyl (benzilic acid β-diethylaminoisopropyl ester hydrochloride), Aprophene (diphenylpropionic acid β-diethylaminoethyl ester hydrochloride), Tiphen (thiodiphenylacetic acid β-diethylaminoethyl ester hydrochloride), Diprophene (thiodiphenylacetic acid β-dipropylaminoethyl ester hydrochloride), IEM-268 (diphenylacetic acid β-dimethylaminoisopropyl ester hydrochloride), Pentaphene (phenylcyclopentacarbonic acid β-diethylaminoethyl ester hydrochloride), thioester 22 (thiobenzilic acid β-diethylaminoethyl ester hydrochloride) and Tropacine (diphenylacetic acid tropinic ester hydrochloride).

The force of the effects induced by the anesthetic and hypnotic substances was determined from the degree of decrease in the animal's motor activity as well as from the duration of the sleep or anesthesia; we used the so-called lateral position of the animals as an index of the onset and duration of the sleep or anesthesia, by which we mean the period during which the animals were unable to turn over from their sides to their stomachs and take the position most usual to them.

Each experiment was performed on two different groups of animals simultaneously, each group containing 10-20 animals; one of these groups received only the anesthetics (control group), while the other was given the same dose of anesthetic 5-10 minutes after the administration of the central cholinolytics. Experiments using anesthetics in combination with the central cholinolytics were also conducted with rabbits; in these experiments, we determined the general condition of the animals and recorded the bioelectric activity of the brain.

RESULTS

Experiments on Mice. Chloral hydrate, intraperitoneally injected in doses of 250 mg/kg, decreased the motor activity of some animals (35%) and induced sleep ("lateral position") in 3-5 out of 20 mice. In the control group of 100 mice, the average duration of the "lateral position" equaled 10 minutes. In a dose of 300 mg/kg, chloral hydrate induced a hypnotic effect lasting 15-30 minutes in all the control animals.

The central cholinolytics (listed above), injected intraperitoneally in doses of 5-10 mg/kg, had no apparent effect on the general behavior and condition of the animals. When 250 mg/kg chloral hydrate was injected on a background of the action of central cholinolytics, we observed a considerable increase in the period of decreased motor activity in the experimental animals and an intensification of chloral hydrate's anesthetic effect: All or the majority of

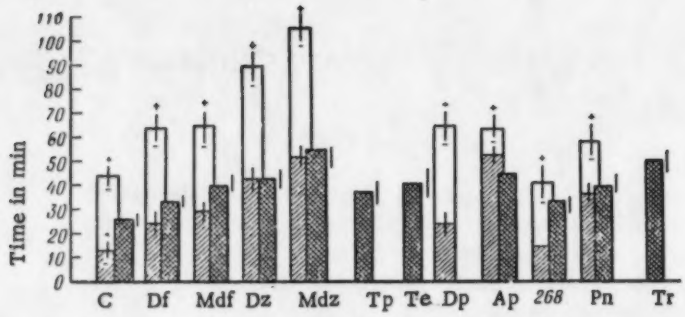

Fig. 1. Influence of central cholinolytics on the hypnotic effect of chloral hydrate. (Average data compiled from 20 observations in each experiment and 220 observations in the control.) White colums) time (in minutes) of reduced motor activity; diagonally striped columns) duration of "lateral position" of animals after intraperitoneal administration of 250 mg/kg chloral hydrate; cross-hatched columns) duration of "lateral position" of animals after intraperitioneal administration of 300 mg/kg chloral hydrate. C) control; Df) Difacyl; Mdf) Methyldifacyl; Dz) Diazyl; Mdz) Methyldiazyl; Tp) Tiphen Te) thioester 22; Dp) Diprophene; Ap) Aprophene; 268) preparation IEM-268; Pn) Pentaphene; Tr) Tropacine (given intraperitoneally in a dose of 5 mg/kg 10 minutes before the administration of chloral hydrate in both cases).

the experimental animals which had received chloral hydrate and a central cholinolytic were in the "lateral position" for 20-50 minutes. The most intensified hypnotic effects were observed when chloral hydrate was combined with Methyldiazyl, Aprophene or Tropacine (Fig. 1).

In the experiments in which chloral hydrate was used in a dose of 300 mg/kg on a background of the action of central cholinolytics, the same definite intensification of the hypnotic effect as compared with that in the control was observed. Although the two different doses of chloral hydrate (250 and 300 mg/kg) induced "lateral positions" of different durations (10 and 25 minutes) in the control, the duration of the hypnotic effect induced by these two doses of chloral hydrate in combination with the central cholinolytics, used in the same dose in either case, was about the same, i.e. 2-5 times stronger than in the control (see Fig. 1). We consider this evidence that central cholinolytics can potentialize the action of hypnotics.

Barbamyl was tested in one dose, 100 mg/kg, administered to the mice intraperitoneally; in the control animals, this dose induced partial anesthesia lasting more than 60 minutes. If central cholinolytics were administered (5 mg/kg) before the Barbamyl, the latter's effect became almost twice as strong (Fig. 2). Barbamyl's effect was still further intensified by the administration of the cholinolytics in a higher dose (10 mg/kg). It was interesting that although the cholinolytics did not induce a hypnotic effect when used alone in this dose, the hypnotic effect of Barbamyl was approximately doubled, i.e., increased according to the increase in the cholinolytic dose.

Hexenal and thiopental were also injected intraperitoneally in doses which did not themselves induce a hypnotic effect (10 and 5 mg/kg). As a rule, Hexenal and thiopental, administered in these doses 5-10 minutes after the administration of the central cholinolytics, induced anesthesia lasting 5-30 minutes in all the experimental mice. We include the results of the experiments with Hexenal by way of example (see Table).

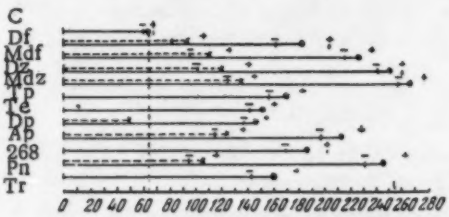

Fig. 2. Change in duration of "lateral position" of mice effected by central cholinolytics in various doses. Solid line) average duration (in minutes) of "lateral position" of mice (20 in each experiment and 180 in control) given 100 mg/kg Barbamyl intraperitoneally 10 minutes after the central cholinolytics (used in a dose of 10 mg/kg); dotted lines) the same on a background of the action of the central cholinolytics injected intraperitoneally in a dose of 5 mg/kg. Same abbreviations used for the preparations as in Fig. 1.

Potentialization of Hexenal's Hypnotic Effect by Central Cholinolytics (5mg/kg)

Preparation	Duration of anesthesia (in minutes) animal number										M	±m	T
	1	2	3	4	5	6	7	8	9	10			
Hexenal (10 mg/kg)	0	0	0	0	0	0	0	0	0	0	0	—	—
Difacyl and Hexenal	6	7	9	14	13	7	14	12	12	12	10,3	3,9	2,6
Methyldifacyl and Hexenal	5	5	13	17	9	10	9	19	18	27	13,2	5,1	2,6
Diazyl and Hexenal	8	12	15	15	27	7	9	16	21	24	15,4	5,8	2,7
Tiphen and Hexenal	8	7	7	9	6	5	7	7	6	8	7,0	2,5	2,8
Methyldiazyl and Hexenal	19	18	17	46	29	20	18	21	24	21	23,4	8,5	2,8
Thioester 22 and Hexenal	17	16	17	14	12	10	10	13	7	36	15,2	6	2,5
Aprophene and Hexenal	12	15	15	25	14	22	6	33	32	6	18,0	7	2,6
Pentaphene and Hexenal	7	38	9	7	54	9	10	19	7	9	17,9	8	2,2
Tropacine and Hexenal	20	14	14	12	22	7	15	8	20	21	15,8	5,6	2,8

Experiments on Rabbits. The influence of central cholinolytics on the effects of hypnotics and anesthetics was studied on rabbits in two types of experiments. In one set of experiments, the force of the anesthetic effect was judged according to the duration of the "lateral position", as in the experiments on mice; in the other set, it was judged according to change in the bioelectric activity of the brain.

The intravenous injection of Hexenal (10 mg/kg) and thiopental (5 mg/kg) induced partial anesthesia lasting 1-4 minutes in 2-3 out of 10 rabbits. The administration of these substances 5 minutes after the administration of central cholinolytics induced an anesthetic effect lasting 5-30 minutes.

Electroencephalographic investigations, performed on rabbits given subanesthetic and anesthetic doses of chloral hydrate, Barbamyl, Hexenal and thiopental and the central cholinolytics in combination with these substances, confirmed the data obtained in the previous experiments on mice and rabbits regarding the potentializing influence of central cholinolytics on the effects of hypnotics and anesthetics.

The intravenous injection in the specified doses of chloral hydrate (100 mg/kg), Barbamyl (35 mg/kg), Hexenal (15 mg/kg) and thiopental (10 mg/kg) induced partial anesthesia of varying duration. At this time, the electroencephalogram (EEG) showed the changes characteristic of this condition: disappearance of the fast potentials, development of slow, high-amplitude waves and, if there was a considerable degree of anesthesia, general inhibition of the bioelectric activity of the brain (Fig. 3, 2).

In subanesthetic doses (75 mg/kg chloral hydrate, 25 mg/kg Barbamyl, 10 mg/kg Hexenal), these preparations did not induce a hypnotic or anesthetic effect; the animals remained lively, and the EEG showed no signs of inhibition of the brain's bioelectric activity (Fig. 3, 4).

Although the central cholinolytics, administered intravenously in doses of 1-5 mg/kg, caused definite changes in the spontaneous bioelectric activity of the brain (Fig. 3, 6), i.e., the appearance of slow waves and a decrease of high-frequency potentials, they were not the changes characteristic of a condition of sleep, especially since the behavior or the rabbits showed no outward signs of sleep.

Small doses of the hypnotics and anesthetics administered intravenously after the central cholinolytics caused sleep and partial anesthesia, and the EEG (Fig. 3, 7) showed changes very similar to those observed with the use of the larger doses of the hypnotics and anesthetics.

In the investigations carried out on mice and rabbits, we obtained statistically authentic data to the effect that central cholinolytics (Difacyl, Methyldifacyl, Aprophene, Pentaphene, Tropacine, etc.) in the specified doses potentialize the hypnotic effect of anesthetics and hypnotics. This was demonstrated by the fact that the cholinolytics, which had no hypnotic effect when used alone, promoted the development of sleep and partial anesthesia when combined with doses of the hypnotics and anesthetics too small to induce a hypnotic effect by themselves (see Figs. 1-3).

This position is further confirmed by the fact that one specific dose of a hypnotic (100 mg/kg Barbamyl, for example) could produce effects of different force and duration depending on the dose of the cholinolytics administered before it. The administration of the cholinolytics in a double dose almost doubled the duration of the sleep induced by Barbamyl, although the cholinolytics themselves had no hypnotic affect in these doses.

The clearly expressed intensifying influence of the central cholinolytics on the hypnotic and anesthetic effects of Hexenal, thiopental, chloral hydrate and Barbamyl is closely connected with the central cholinolytic activity of the preparations. This is indicated by the difference in the effect induced by preparations IÉM-268 and Diprophene from that induced by Diazyl and Aprophene. The cholinolytic effect of the former on the central cholinergic systems is weak, and their potentializing influence on the hypnotic effect is slight.

The greatest potentialization of the effect of hypnotics and anesthetics occurred under the influence of Diazyl, Methyldiazyl, Tropacine and Aprophene, preparations which

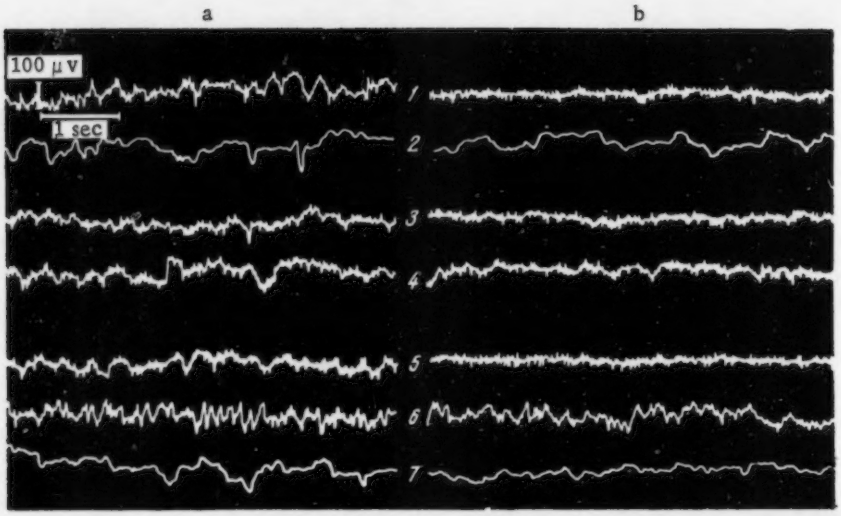

Fig. 3. Potentialization of Barbamyl's effect on the bioelectric activity of a rabbit's brain under the influence of Diazyl. a) EEG of cerebral cortex; b) EEG of optic thalami; 1, 3, 5) norm; 2) EEG taken 5 minutes after the intravenous injection of 35 mg/kg Barbamyl; 4) the same after 25 mg/kg Barbamyl; 6) EEG taken 5 minutes after the intravenous injection of 5 mg/kg Diazyl; 7) EEG taken 5 minutes after the intravenous administration of 25 mg/kg Barbamyl on a background of the action of the central cholinolytic Diazyl.

have a pronounced cholinolytic effect on the central cholinergic structures.

Although comparison of the effects of the various preparations shows some differences as to force, the differences were only found to be authentic in the case of Methyldifacyl and Methyldiazyl in relation to Difacyl and Diazyl, in the case of Aprophene, Pentaphene and Tropacine in relation to Difacyl, Diprophene and IÉM-268 and in the case of all these preparations in relation to the data of the control experiments.

These investigations of the potentializing influence exerted by central cholinolytics on the effects of hypnotics and anesthetics confirmed the rules demonstrated earlier regarding the link between structure and effect in a series of central cholinolytics. For example, there is a direct relationship between the increase in the central cholinolytic activity and the potentializing influence on hypnotic and anesthetic effects of preparations belonging to the Difacyl series as additional radicals are introduced into the structural formula. The transformation of Difacyl into Aprophene and Diazyl or of Diazyl and Difacyl into the β-methyl derivatives is a good example of this.

Therefore, on the basis of the experimental data obtained, one can conclude that preparations of the central cholinolytic group can potentialize the action of hypnotics and anesthetics. Central cholinolytics can be recommended for practical use in enhancing the effect of hypnotics and anesthetics (so that a smaller dose of the anesthetic is required to produce anesthesia of adequate depth).

SUMMARY

Methyldiazyl, Diazyl (benacthyzine), Aprophene, Pentaphene (Parpanit), Defacyl (Trasentine), Menthyldifacyl, Tropacine, Tiphen and thioester 22, preparations pertaining to the group of central cholynolytics, can potentialize the action of hypnotics and anesthetics (chloralhydrate), Barbamyl, Hexenal and thiopental). The enhanced action of hypnotics and anesthetics under the influence of the above complex esters is caused by the central cholinolytic properties of these preparations. There is a direct relationship between the degree of hypnotic potentialization and the intensity of the cholinolytic properties of these substances: The most potent central cholinolytics (Methyldiazyl, Diazyl, Pentaphene, Aprophene and Tropacine) have the strongest intensifying effect on hypnotic and anesthetic action.

Hence, central cholinolytics may be recommended for practical use in enhancing the action of hypnotics and anesthetics.

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A STUDY OF SPINAL CORD INHIBITION PROCESSES AND REFLEX EXCITABILITY UNDER CONDITIONS OF ANESTHESIA

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(Presented by V. V. Zakusov, Active Member of the AMN SSSR)
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The works of I. P. Pavlov, N. E. Vvedenskii and A. A. Ukhtomskii contain valuable ideas and facts which make it possible to approach an explanation of the physiological nature of anesthesia. The physiological aspect of anesthesia has been most profoundly investigated by N. E. Vvedenskii [1], P. V. Makarov [5] and V. V. Zakusov [4]. On the basis of experimental data obtained on nerve-muscle preparations, N. E. Vvedenskii [1] described anesthesia as a condition of parabiosis, the essential feature of which is "that it affects all the contiguous parts simultaneously and is a more or less stable and constant state."

The purpose of this work was to study the changes which occur in the cerebrospinal reciprocal inhibition and reflex excitability processes of warm-blooded animals under conditions of anesthesia.

METHODS

The experiments were performed on spinal cats and on some decerebrate cats. We used ether or chloroform to produce inhalation anesthesia and Pentothal, Nembutal and Hexenal to produce intravenous anesthesia. We investigated the changes which occurred during anesthesia in the reciprocal inhibition and reflex excitability of the flexor and extensor muscles of the posterior extremities. The anterior tibialis muscle was used to determine change in the excitability of the flexor reflex and in the contralateral inhibition. The reflex contractions of this muscle were induced by single inductive stimulations of the popliteal nerve of the same paw, while the inhibition was induced by stimulating the same nerve in the opposite paw. We used the gastrocnemius muscle to study the excitability of the extensor reflex and the ipsilateral inhibition. The reflex contractions of this muscle were induced by single inductive stimulations of the posterior root of the 7th lumbar segment, and the inhibition was induced by similar stimulations of the deep peroneal nerve of the same extremity.

Reflex excitability was evaluated according to height of the recording produced on a kymograph by the contractions of the experimental muscle in response to a single inductive stimulation of its sensory nerve. The degree of inhibition was estimated from the height of the recording produced by the contractions of the same muscle on a background of an inhibitory discharge given 40-60 milliseconds before the reflex discharge in the case of contralateral inhibition and 8-10 milliseconds before in the case of ipsilateral inhibition. The inhibition was most apparent at the specified intervals. The proper intervals between the stimuli were selected with the aid of a Helmholtz's pendulum.

In some experiments, the contraction of the tibialis muscle was induced by stimulation of the descending lateral cerebrospinal tract. For this purpose, the spinal cord was exposed at the level of the 5th lumbar segment, and special bipolar electrodes were inserted from the dorsal surface of the spinal cord in such a way that their non-insulated ends were located in the specified tract. The electrodes were silver wires about 30 μ in diameter, sealed in a glass capillary. In this case, the inhibitory stimulus was produced by stimulating the posterior roots of the opposite side of the 7th lumbar segment.

RESULTS

The investigations showed that the state of anesthesia induced by the inhalation of ether or chloroform, or by the intravenous injection of the barbiturates, intensified the contralateral inhibition and reduced the reflex excitability of the flexor muscles of the posterior extremities. Fig. 1 gives a myogram of an experiment which illustrates the changes which occurred with the intravenous injection of 2 ml of a 2,5 % Pentothal Sodium solution into a spinal cat weighing 1.7 kg. The myogram shows that the inhibition process became stronger in the initial stage of the anesthesia, before any change occurred in the height of the reflex contractions of the muscle (see first and second pairs of recordings after arrow); subsequently, as the anesthesia deepened and reflex excitability decreased, the intensification of the inhibition processes continued. Therefore, the intensification observed of contralateral inhibition cannot be accounted for by the weakening of the reflexes.

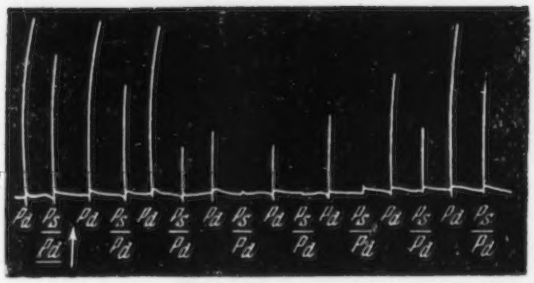

Fig. 1. Change in the contralateral inhibition and excitability of the flexor reflex during anesthesia. Pd) height of contractions of right anterior tibialis muscle in response to a single stimulation of right deep popliteal nerve; $\frac{Ps}{Pd}$) the same on a background of a single discharge from the left popliteal nerve (40 milliseconds previously). The arrow represents the intravenous injection of 2 ml of 2.5% Pentothal Sodium into a spinal cat weighing 1.7 kg. Duration of investigation - 45 minutes.

Intensification of the inhibition processes during anesthesia was also characteristic of the experiments in which the contraction of the tibialis muscle was induced by single inductive discharges of the descending lateral cerebrospinal tract and the inhibition, by single stimulations of the opposite posterior root. The change which occurred in the ipsilateral inhibition and reflex excitability of the extensor muscle is shown in Fig. 2. This type of reflex inhibition, unlike the contralateral inhibition, was observed to become briefly weaker in the initial phase of the anesthesia before it became stronger. In these experiments, as in the preceding ones, the intensification of the inhibition occurred before the decrease in the reflex excitability (see Fig. 2, second pair of recordings after arrow), so that it could not be due to the weakening effect of anesthesia on the reflexes.

The changes which developed in the reciprocal inhibition and reflex excitability during anesthesia were reversible as the animal emerged from the condition of anesthesia.

In contralateral and ipsilateral inhibition, the impulses are known to pass along the intercalary neurons. It is therefore natural to suppose that they become weaker during anesthesia when the transmission of stimulation in the polysynaptic paths is blocked. However, although the inhibitory impulses do become somewhat weaker in the polysynaptic paths under conditions of anesthesia, the conditions created by the anesthesia in the motor cells of the spinal cord are extremely favorable for the manifestation of stronger than normal inhibition of reflex discharges. Our previous investigations [7, 9] showed the decrease in the functional mobility (prolongation of chronaxy) and increase in the accommodation rate (decrease in the constant λ) of the spinal cord motor cells during anesthesia to be such conditions. In [6], P. E. Motsnyi established that functional changes of the same nature also develop in the spinal cord motor cells under conditions of inhibition.

Therefore, the functional changes which develop in the motor centers of the spinal cord under conditions of inhibition and anesthesia are the same in nature. In a previous work [9], we showed that these changes in the motor centers of the spinal cord could be cumulative with inhibition of a background of anesthesia. This evidently is the reason for the intensification of the inhibition processes under conditions of anesthesia.

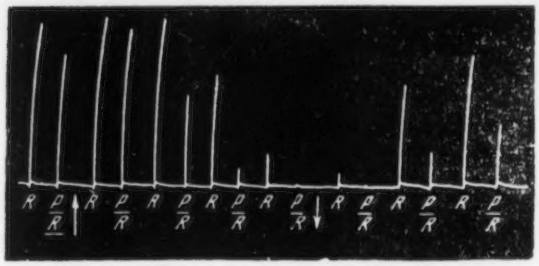

Fig. 2. Change in the ipsilateral inhibition and excitability of the extensor reflex during anesthesia. R) height of contractions of gastrocnemius muscle in response to a single stimulation of the posterior root (L_7); $\frac{P}{R}$) the same on a background of a single impulse from the right deep peroneal nerve (10 milliseconds previously). (Arrows indicate beginning and end of ether inhalation. Inhalation duration - 4 minutes.)

As to the changes in the reflex excitability of the spinal cord during anesthesia, the experiments showed that the changes are different for the flexor and extensor reflexes of the posterior extremities. The decrease in the reflex excitability of the extensor muscles was preceded by a brief phase of facilitation of the reflex. In the case of the flexor muscles, the phase of facilitation of the reflex was absent; the decrease in reflex excitability paralleled the increase of the anesthesia. These differences are evidently due to the higher lability indicated by N. E. Vvedenskii and A. A. Ukhtomskii [2] of the extensor muscle centers as compared with flexor centers.

The "rule of optimal physiological lability for excitability" [3] can be applied to explain how reflex excitability depends on change in lability. According to this rule, excitability is highest with a certain mean value of physiological lability. When the lability becomes either higher or lower than this optimal level, the excitability of nervous tissue decreases and the conduction of stimulation is hampered. Fig. 3 shows N. V. Golikov's rule [3] as applied to explain the dependency of change in the excitability of the nerve centers of flexor and extensor muscles on the change in their lability during anesthesia. Anesthesia lowers the lability of the spinal cord motor centers [7, 9]; in the centers of the flexor reflexes (located in left part of diagram in Fig. 3), which are formations less labile than the extensor centers, the excitability decreases parallel to the lability, and there is no phase of reflex facilitation. In the centers

of the extensor reflexes (located in the right part of the diagram in Fig. 3), the more labile formations, the decrease in lability which occurs under the influence of anesthesia passes through the optimum point at which excitability is highest. Therefore, an initial facilitation of the reflex is observed in the early stages of the anesthesia. As the anesthesia deepens and the lability of the centers falls below the optimal value, the excitability also decreases.

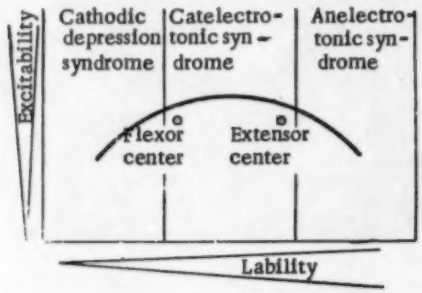

Fig. 3. "Rule of optimal physiological lability for excitability" (N. V. Golikov [3]), used to illustrate how change in the excitability of the motor centers of flexor and extensor muscles depends on change in their lability during anesthesia.

All the experimental anesthetics caused these reversible changes of decreased reflex excitability and increased reciprocal inhibition; the duration and intensity of the changes, however, depended on both the dosage and the physiological activity of the anesthetics. The intensification of reciprocal inhibition during anesthesia, together with the decreased functional mobility and increased accommodation rate of the spinal cord motor cells (respectively, prolonged chronaxy and decreased constant λ) observed earlier by us under conditions of anesthesia [7-9] and by P. E. Motsnyi [6] with reciprocal inhibition, testifies that the functional changes which develop under conditions of inhibition and anesthesia are similar in nature and fits in well with N. E. Vvedenskii's doctrine of parabiosis.

SUMMARY

Myography was used on spinal cats to investigate the changes in the reciprocal inhibition and reflex excitability of extensor and felxor muscles during anesthesia. The reciprocal inhibition was intensified during anesthesia induced by ether or chloroform inhalation as well as during that induced by the intravenous injection of sodium pentothal or nembutal.

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*See C. B. translation.

THE ANTIGENIC STRUCTURE OF THE ROUS SARCOMA COMMUNICATION I. DETECTION OF THE SPECIFIC ROUS SARCOMA ANTIGEN BY THE PRECIPITATION TEST IN AGAR

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(Presented by Active Member AMN SSSR L. A. Zil'ber)
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The precipitation test in agar has been used successfully in recent years in the study of the antigenic structure of multi-component systems [12], including tissues affected by viruses [9, 10, 14].

In the present research we attempted to use the method for the analysis of the antigenic structure of the Rous sarcoma—a tumor of fowls caused by a virus.

In the first stage of the investigation the antigenic composition of the Rous sarcoma was compared with the antigenic composition of the organs of healthy fowls. For purposes of comparison various organs and tissues of healthy fowls were used, as follows: liver, kidney, spleen, lung, ovaries, muscles, subcutaneous cellular tissue and blood serum, and in addition a transplanted fowl sarcoma initially induced by methylcholanthrene, and a culture of fibroblasts.

METHOD

The work was undertaken with a strain of the Rous sarcoma virus obtained from Carr's laboratory. This strain of the virus has been transmitted for many years through fowls to the White Leghorn breed. The strain MKh-659 of methylcholanthrene sarcoma was obtained from A. M. Dyad'kova (Institute of Oncology, Leningrad) and also transmitted through fowls of the same breed.

Normal organs and tissues were taken from similar healthy fowls. Fibroblasts were obtained by I. S. Irlinym from 9-day chick embryos and subsequently grown in tissue culture.

As antigens we used extracts (pH = 7.0-7.2) of tissues ground with sand in physiological saline (1:5 - 1:8). Hyaluronidase was added to the Rous sarcoma extract in a proportion of 1:100. In order to remove coarse particles of tissue and sand, the extracts were centrifuged at the speed of 2500 rpm for 30 minutes.

The proteins were determined by the quantitative biuret reaction, and in some experiments by Kjeldahl's method. Theprotein content of theRous sarcoma extracts varied between 7 and 11 mg/kg, and that of the extracts of organs of healthy fowls and of sarcoma MKh-659 tissue from 12-15 to 20-30 mg/ml.

Antisera to tumor and normal fowl tissues were obtained by immunization of rabbits in accordance with the following scheme.

The first cycle of immunization consisted of three injections: the first a subcutaneous injection of 5 ml of extract with a lanolin depot, and the second and third intramuscular injections of 10 ml of extract. The interval between the first and second injections of the first cycle of immuniaztion was 14 days, and that between the second and third injections 7 days. The second cycle of immunization consisted of two intramuscular injections, each of 10 ml of extract, at an interval of 7 days. The interval between the first and second cycle of immunization was 30-40 days.

In accordance with this immunization scheme we obtained six rabbit sera against Rous sarcoma and two antisera against normal fowl liver, kidney, spleen, lung and muscle, normal fowl serum* and sarcoma MKh-659.

In the experiments both original and exhausted antisarcoma sera were used. Exhaustion was carried out fractionally, in several stages: At first, normal fowl serum was added, then a mixture of healthy fowl's organs (liver, kidney, spleen and muscle), and thirdly, an extract of sarcoma MKh-659. At each stage of the exhaustion procedure the mixture of antisarcoma serum with the exhausting antigens was incubated at 37° for 1-1.5 hours and at 4° for 2-4-16 hours. The precipitates formed on addition of antigens from normal tumor tissues were separated by centrifugation at 2000-2500 rpm for 5-10 minutes. As a rule the exhaustion of 1 ml of antiserum against Rous sarcoma required a total of 20-25 mg of protein of the exhausting antigens. The exhausted antisarcoma serum was investigated by means of the precipitation test in agar for completeness of exhaustion. If it continued to react with any of the antigens in a dose known to be greater than the dose of antigen used for exhaustion of the serum, further exhaustion in relation to this antigen was undertaken. Since the serum became diluted in the process of exhaustion, it was concentrated to

* During immunization with serum, 5 ml of normal fowl serum was given at each of the 5 injections.

the initial volume in a cellophane bag, cooled in a current of air from a fan. After concentration, the serum was dialyzed against physiological saline. The concentration and dialysis were carried out at a temperature of 6-8°.

The antigens and antisera were tested by the precipitation reaction in agar, in a micro-modification, and by the ring-precipitation test. The ring-precipitation test was performed in the usual manner. In the experiments we used 19 immune and 18 normal rabbit sera, 9 specimens of antigens from Rous sarcoma and 5 from sarcoma MKh-659, 10 different antigens each from the liver, kidney, spleen, lungs and muscles of healthy fowls and 10 normal fowl sera.

RESULTS

A comparison between antigens from the Rous sarcoma and antigens from normal fowl's kidney is made in Fig. 1. The original antisarcoma serum gave several precipitation rings, characteristic of both Rous sarcoma and of normal fowl's kidney. Besides the general precipitation spectrum, an additional band of precipitation with antigen from Rous sarcoma was constantly observed. The same picture was observed with antigens from the liver, spleen, lungs, subcutaneous cellular tissue and ovaries of a healthy fowl, with normal fowl serum and with antigens from fibroblasts and from sarcoma MKh-659.

Only one precipitation band with antigen from Rous sarcoma appeared with the exhausted antisarcoma sera. This precipitation band was not observed with any antigen from healthy fowl's organs or from sarcoma MKh-659 (Fig. 2), despite the fact that the normal antigens and the antigens from sarcoma MKh-659 were present in the majority of experiments in a protein concentration two to three times greater than Rous sarcoma antigen.

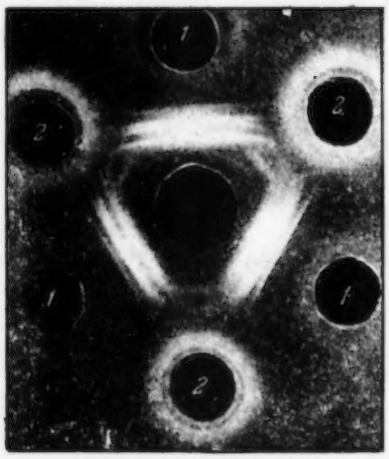

Fig. 1. Comparison of the antigenic composition of Rous sarcoma and of normal kidney. In the center) original serum No. 863 against Rous sarcoma. 1) antigens from Rous sarcoma tissue; 2) antigens from normal fowl's kidney tissue.

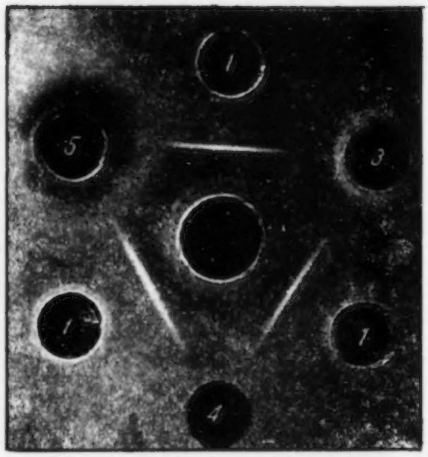

Fig. 2. Detection of specific Rous sarcoma antigen with exhausted antisarcoma serum. In the center) exhausted antiserum No. 863. Exhaustion carried out with normal fowl serum and a mixture of extracts of normal fowl's organs and of a methylcholanthrene tumor. 1) antigens from Rous sarcoma tissue; 3) antigens from tissue of tumor MKh-659; 4) normal serum; 5) antigens from healthy chick's muscle.

The specific Rous sarcoma antigen was also detected when the conditions of the test were even more exacting [1, 4].

One such experiment is illustrated in Fig. 3, from which it can be seen that the precipitation band formed by the specific Rous sarcoma antigen and by specific antibodies against this antigen rests with its ends in the hole containing a heterologous system.

This antigen, consequently, is absent from extracts of normal fowl tissues, and antibodies against it are present only in antiserum against Rous sarcoma.

We were then confronted with the problem: Is the specific Rous sarcoma antigen an isoantigen? In order to exclude this possibility, we induced the tumor not by a cell suspension but by a filtrate of a Rous sarcoma, and compared its antigenic composition with the antigens from the organs of the same fowl. In these experiments too, in which the possible participation of isoantigens was excluded, the specific Rous sarcoma antigen was detected just as clearly as in the preparations demonstrated above.

The precipitation test in agar thus revealed the presence of a specific Rous sarcoma antigen, which was not an isoantigen.

Specific Rous sarcoma antigen was detected in four of the six original sera and in five of the six exhausted antisarcoma sera (exhaustion facilitates the detection of the specific antigen).

Minimal Doses of Antigens in mg / ml of Protein[1] to Produce a Ring-Precipitation Reaction

No. of rabbit	Antigens / Antisera	From liver tissue	From kidney tissue	From spleen tissue	From lung tissue	From muscle tissue	From normal fowl serum	From tissue of filterable Rous sarcoma	From tissue of methylcholanthrene tumor
852	Against liver tissue	0,01	0,018	0,036	0,016	0,064	0,008	0,01	0,018
853		0,01	0,018	0,036	0,016	0,128	0,008	0,02	0,018
854	Against kidney tissue	0,02	0,009	0,036	0,016	0,128	0,008	0,02	0,018
855		0,01	0,009	0,036	0,008	0,128	0,004	0,01	0,035
856	Against spleen tissue	—	—	—	—	—	—	—	—
857		0,04	0,018	0,036	0,008	0,064	0,004	0,02	0,035
858	Against lung tissue	0,01	0,009	0,036	0,016	0,26	0,008	0,02	0,035
859		0,01	0,018	0,036	0,008	0,26	0,004	0,02	0,035
889	Against muscle tissue	0,01	0,006	0,01	0,004	0,0007	0,006	0,003	0,005
990		0,01	0,006	0,01	0,004	0,0007	0,006	0,003	0,005
860	Against normal fowl serum	0,02	0,009	0,036	0,016	0,064	0,004	0,01	0,035
861		0,02	0,009	0,036	0,008	0,028	0,004	0,02	0,035
862	Against tissue of filterable Rous sarcoma	0,02	0,009	0,036	0,008	0,033	0,008	0,005	0,018
863		0,02	0,009	0,036	0,016	0,033	0,004	0,005	0,018
887	Against methylcholanthrene tumor	0,018	0,025	—	0,054	0,014	0,006	0,006	0,015
888		0,018	0,025	—	0,027	0,014	0,006	0,006	0,015

[1]The protein content of the antigens was determined by Kjeldahl's method.

Antisera against normal fowl's organs (liver, kidney and muscle) and against normal fowl serum revealed components in homologous antigens that were not present in Rous sarcoma.

The 18 sera taken from the rabbits before immunization in general gave no precipitation reactions with either extracts from healthy fowl's organs or extracts from fowl sarcomas.

Side by side with the precipitation test in agar, we also used the crossed ring-precipitation test for differentiation of the specific Rous sarcoma antigen.

In the ring-precipitation test we used the same antigens and the same antisera as in the precipitation test in agar. The results of one crossed ring-precipitation experiment with antisera from the second cycle of immunization are given in the table.

All the antisera—antitumor and against healthy fowl's organs—reacted more strongly with normal fowl serum than with their own homologous antigen. Consequently, contamination with serum proteins, which are always present in the tissues to be tested, masks the antigenic differences between the individual organs and makes the results unreliable.

The antigens from muscle tissue are an exception.

The present work is the first stage in a study which we are undertaking of the antigenic structure of the Rous sarcoma. The specific antigen of this tumor was found earlier by means of the following reactions: virus neutralization [11, 13], anaphylaxis with desensitization [3, 8], complement fixation [6, 11], passive hemagglutination [5, 7] and precipitation in agar [2]. Most authors considered that this antigen is a virus antigen. In order to study the antigenic structure of the test tissue, the method of precipitation in agar shows great promise, for in practice it will reveal the complete antigen spectrum of the tissue and enable each antigen to be analyzed separately.

When Rous sarcoma is being compared with a homologous normal tissue, difficulty arises in the choice of an ade-

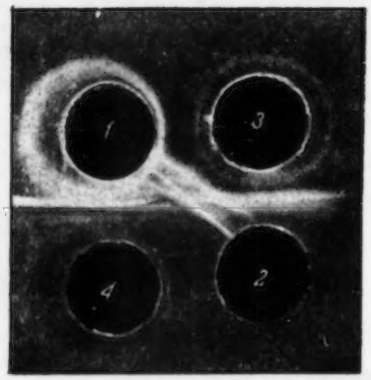

Fig. 3. Comparison of the systems: sarcoma-antisarcoma serum and lung–anti-lung serum. 1) original serum No. 858 against healthy fowl's lung tissue; 2) antigens from healthy fowl's lung tissue; 3) original serum No. 863 against Rous sarcoma tissue; 4) antigens from Rous sarcoma tissue.

quate control. Usually normal fowl serum and muscle are used as a control to this tumor, because this sarcoma grows in the muscle of the fowl [3, 8]. We consider that it is insufficient to restrict the comparison to muscle and normal fowl serum, for in addition to muscle tissue, the sarcoma is mainly composed of fibroblast-like cells, degenerative elements and an amorphous, mucoid substance. Our experiments actually showed that the sarcoma tissue differs from muscle not only in its specific antigen, but also in other antigens present in the organs of healthy fowls.

Besides muscle, we therefore used fibroblasts from chick embryos, grown in tissue culture, spleen, liver, kidney, lungs and normal fowl serum.

The use of the proteins of normal fowl serum as control preparations and, in particular, for the exhaustion of the immune sera in the Rous sarcoma, is of special importance. The serum proteins are extremely powerful antigens, which often mask the weaker precipitation lines of the tissue antigens, and the removal of antibodies against these proteins is therefore an essential condition for the detection of tissue antigens.

In this connection it must be pointed out that failure to differentiate the specific Rous sarcoma antigen by means of the ring-precipitation test is presumably due to the presence of serum proteins in the organs of healthy fowls.

A distinctive feature of the growth of the filterable sarcoma is its mucous character. In addition to the control preparations listed above, we therefore tested a tissue rich in tissue polysaccharides, namely the subcutaneous cellular tissue of a healthy chick. Finally, the last control preparation was antigen from sarcoma MKh-659. We consider that a combination of the antigens of all the selected tissues and organs creates a more adequate control to the Rous sarcoma than the antigens of each organ and tissue taken separately.

In work with non-inbred animals, there is always the possibility of detecting isoantigen instead of the specific tumor antigen.

This possibility was excluded in our experiments by comparing a tumor induced by a filtrate, and consequently arising from the tissues of the host, with the organs of the same fowl. In this case too, a specific antigen which was not an isoantigen was revealed.

By means of the precipitation test in agar we were thus able to detect an antigen which was not present in the organs and tissues of a healthy fowl, nor in sarcoma MKh-659.

Details concerning the nature of the specific Rous sarcoma antigen will be given in later communications.

SUMMARY

With the aid of the precipitation reaction in agar and the ring-precipitation test the authors compared the antigenic composition of Rous sarcoma with that of the organs of healthy chickens (liver, kidney, spleen, subcutaneous cellular tissue, muscles, etc.), transplantable chicken sarcoma MKh-659 (primarily induced with methylcholanthrene), and normal chicken serum. Evidence was obtained that it is possible to detect the specific antigen in Rous sarcoma extracts only with the aid of precipitation in agar, while the ring precipitation reaction does not permit differentiation of this antigen.

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THE INNERVATION OF THE TRANSPLANTED BROWN-PEARCE TUMOR OF RABBITS

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(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
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The problem of the innervation of malignant tumors in man and animals has long attracted the attention of researchers. The study of this problem gives a deeper understanding of the relationship between the tumor and the host, and is thus of practical as well as theoretical importance. A point of special importance here is the question of the innervation of transplanted tumors, but little reference to it is to be found in the specialized literature. Whereas references to the innervation of experimental tumors number several tens of authors, those dealing with the innervation of transplanted tumors are to be found only very rarely.

The first research in this direction was carried out by Milone [9], who studied the innervation of the rat sarcoma. In this author's opinion, nerve fibers growing into a transplanted tumor, placed between the ends of the divided nerve, bore no relationship to the tumor. M. N. Meissel and L. F. Larionow[8], however, who carried out similar experiments, observed on the other hand that there was a close morphological connection between the nerve cells and the tumor cells, which, in their opinion, implies functional association too. Later, A. G. Chernyakhovskii [5, 6] found nerve endings in intimate contact with tumor cells in an Ehrlich's mouse carcinoma.

In recent years Ukranian workers [1, 2, 3], studying a transplanted M-1 sarcoma of rats and a mouse strain of Crocker's tumor, have described nerve cells in these tumors. At the same time these authors emphasize the presence of marked dystrophic changes in the nerves of these tumors. Ducan and Bellegie [7] described the ingrowth of nerve fibers from the posterior columns and dorsal roots of the spinal cord into a transplanted rat sarcoma No. 319 after inoculation of the tumor in the cerebrospinal canal.

Little or no work has been done on the innervation of the Brown-Pearce tumor. It is therefore necessary to dwell in greater detail on the investigations of Shapiro and Warren [10], who studied the stage of the nerves in 13 transplanted Brown-Pearce tumors in the anterior chamber of the rabbit's eye. The peripheral end of the corresponding sympathetic nerve of the eye was stimulated with the electric current, and the reaction of the vessels was observed visually: blanching of the vessels in the transplant indicated that the vasomotor effect extended to the malignant tumor. Impregnation of the tumors by Bodian's method revealed solitary nerve fibers in the tumor, or else fibers collected in bundles of 10-12 or more in each. These authors particularly stress the presence of nerves on the ninth day after transplantation of the tumor. Since the tumor was transplanted into the anterior chamber of the eye, Shapiro and Warren [10] considered these findings to be important evidence that the nerve fibers are not nerves embroiled by the tumor in the course of its invasive growth. From their investigations these authors concluded that the constriction of the blood vessels of the Brown-Pearce tumor transplants is the result of stimulation of the sympathetic nerve supplying them.

METHOD

Our experiments were carried out on chinchilla rabbits, mainly males, weighing about 2 kg, on which the operation of retroperitoneal injection of 0.05 ml of tumor emulsion into the left adrenal gland was carried out under ether anesthesia. The tumor suspension was prepared in sterile conditions by cutting the tumor finely in a Petri dish by means of Cooper's scissors. The minced tumor was diluted with physiological saline to the required concentration (25%) and filtered through a double layer of gauze. The fluid injected contained no visible tumor particles. Animals were sacrificed after 10, 14, 21, 30 and 40 days. The adrenal glands (right and left) were fixed in AFA mixture by Lavrent'ev's method for one hour, and then transferred to 20% neutral formalin. Sections were cut out to a thickness of 40-50 μm with a freezing microtome, impregnated by Kampo's method and stained with hematoxylin-eosin to determine the situation of the tumor.

RESULTS

A total of 52 left adrenal glands were investigated after primary implantation of a Brown-Pearce tumor, and nerve structures were found in 39 tumors in these glands;

the same number of right adrenal glands was examined, in nine of which metastases were detected microscopically and nerve cells were demonstrated therein.

Nerve fibers were observed among the tumor cells in the left adrenal gland on the tenth day after transplantation of the tumor. They issued from plexuses, gave off branches, curved around the tumor cells on every side and ended on them in the form of thickenings (Fig. 1).

Fig. 1. Fine nerve fibers among tumor cells on the tenth day after inoculation of the tumor in the left adrenal gland. Rabbit No. 1964. Silver impregnation by Kampos' method.

On the 14th day and also on the 21st day after transplantation of the tumor the following picture of innervation of the tumor was observed. At first the nerves usually passed through the cortical layer and then entered the tumor, where breaking up of the bundles took place, the individual fibers being separated from each other by tumor cells. Some fibers in the bundle penetrated into the tumor for a considerable distance, taking a course in the direction from the periphery to the center. Occasionally nerve fibers were also observed in necrotic areas of the tumor. Growth bulbs could be seen very often at the ends of the nerve fibers, together with dichotomous division of the fibers (Fig. 2).

In every case signs of irritation of the nerve fibers were seen, in the form of increased argentophilia, varicose swellings along the course of the nerve fibers and bublar thickenings at their ends. In one case (rabbit No. 603) considerable changes were observed among the fibers situated in the substance of the cortex. Special mention must be made of a case (rabbit No. 514) in which the tumor nodule, situated outside the adrenal gland, partially engulfed the gland and infiltrated into its cortical layer, although nowhere did it reach the substance of the medulla (the tumor was separated from the medulla by a strip of cortex). Nevertheless, here too, nerve fibers were found. Some of them were greatly thickened and argentophilic, others very thin—intermingled on all sides with the tumor cells. There were many such fibers and they could be followed for a considerable distance. On the 30th day, while the distribution of the nerve fibers bore the same character, the development of endings was observed. For instance, some fibers, after undergoing dichotomous division, terminated among the

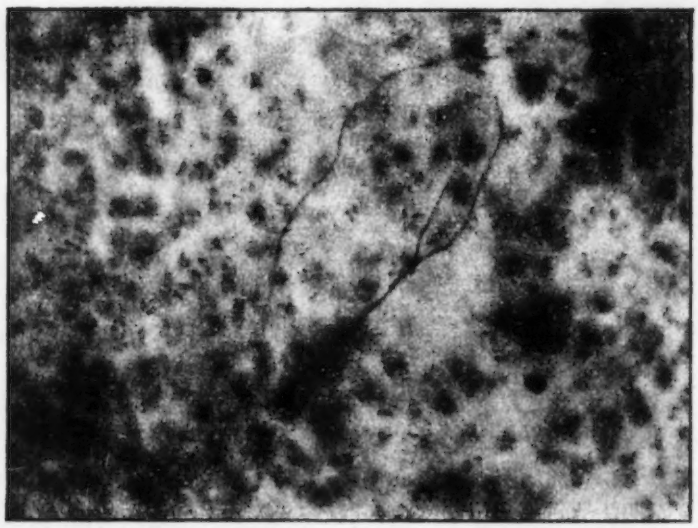

Fig. 2. Ramification of thin nerve fibers in a tumor on the 21st day after inoculation of the tumor in the left adrenal gland. Rabbit No. 603. Microphotograph. Silver impregnation by Kampos' method. Ocular 10 ×, objective 32 ×.

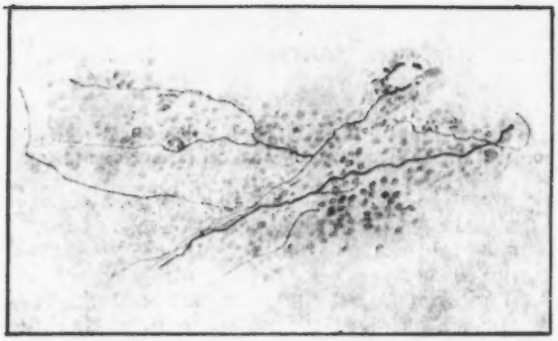

Fig. 3. Bundle of nerve fibers and individual nerve fibers undergoing dichotomous division, terminating amid tumor cells (on the 30th day after inoculation of the tumor in the left adrenal gland). Rabbit No. 192. Silver impregnation by Kampos' method.

tumor cells in the form of pointed endings (Fig. 3), and in isolated cases of pin-head structures.

It must be pointed out, however, that with an increase in the length of time after transplantation of the tumor, the number of nerve fibers in the tumor decreased, especially on the 40th day, evidently on account of necrosis of the tumor and the death of its nervous apparatus. At this stage of development of the tumor, irritation of the nerve fibers was particularly pronounced in the capsule.

In nine cases metastases were present in the right adrenal gland. In two cases metastases were only found histologically. The area affected by the metastasis was different in each case. It may be assumed that the dimensions of the metastasis were dependent on the time of its appearance.

In all cases many nerve fibers were found in the metastases, being arranged haphazardly in the tumors, sometimes in the form of a network. Some fibers were thickened and highly argentophilic. Along with these there were many thin nerve fibers, the latter undergoing dichotomous division. Along the course of the thin fiber varicose swellings could be observed.

When the findings obtained from the study of the nerve formations in the transplanted Brown-Pearce tumor in the rabbit are examined, it is above all essential to note that nerve structures were found in the tumor at different times (10, 14, 21, 30 and 40 days) after transplantation. Under these circumstances the number of nerve cells, and their character, in the tumor transplanted into the afrenal gland differed from the corresponding features of the nervous apparatus in the adrenal medulla. The same findings were observed in the metastases of this tumor in the opposite adrenal gland, into which nerve fibers grew from neighboring nerve trunks.

Our findings do not agree with the statements in the literature [9] that nerve cells, if they are present in tumors, are not in contact with the tumor cells. The microphotograph and the figures illustrate the ingrowth of very thin nerve fibers between the tumor cells. In isolated cases, at the stage of 21-30 days after transplantation of the tumor, when the nerve formations in the tumor were most numerous, nerve endings were also found.

These nerve fibers which grew into the tumor, to form growth bulbs and nerve endings, must therefore be regarded as belonging to the tumor itself.

Particularly noteworthy were the changes affecting the nerve fibers both in the tumor and in the surrounding tissues. Besides the growth bulbs, connected with the growth processes of the nerve fibers, it was possible to see varicosities of the nerve fibers, spherical thickenings and accumulations of neuroplasm, spiral coiling and hyperargentophilia. These changes were manifestations of irritations, and were present to a varying degree depending on the time after transplantation of the tumor. These signs of irritation were most pronounced on the 21st-30th day, when the tumor itself had reached its peak of growth and development, and in many cases had resulted in a fatal outcome. On the 40th day the number of nerve fibers was relatively small on account of the development of necrosis of the tumor.

The results obtained are in full agreement with those of investigations which we carried out [4] on tumors of the salivary glands in man. Although in neither case have exhaustive details been obtained on the functional significance of the nerve formations found in the tumors, the presence of endings of sensory type in human tumors and the appearance of endings very similar to sensory endings in the experimental tumors (see Fig. 3) demonstrate that these structures are indeed the medium by which nervous influences from the tumor are conveyed to the rest of the body.

SUMMARY

The author investigated the innervation of an experimental Brown-Pearce tumor transplanted to the adrenal gland in rabbits. Nerve elements were revealed in the tumor at various times after its transplantation—in 10, 14, 21, 30, and 40 days.

The tumor nerve fibers became most numerous 21-30 days after transplantation and at that time nerve endings were also revealed. Many nerve fibers were found in tumor metastases in the other adrenal gland. Thus, the nerve endings growing in the tumor should be regarded as nerves belonging to the tumor itself.

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CHANGES IN THE ACCESSORY SEX GLANDS OF MALE RABBITS FOLLOWING CASTRATION

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Recently, an increased interest is appearing in the study of hormonal influences on the accessory sex glands. In connection with this it is necessary to compare hormonal alterations with those which arise as a result of castration. However, investigators mainly describe changes in the prostate gland [1-6, 10, 11] and the seminal vesicle [7, 8, 10]. Only a small number of them also devote attention to other glands of the male sexual apparatus [9]. In addition, it is difficult, on the basis of sources in the literature, to arrive at a definite opinion as to whether or not castration causes a proliferation of connective tissue and annexation of inflammation in the accessory sex glands, as the descriptions in this connection have been inconsistent.

These considerations were the impetus for carrying out this work.

METHOD

The experiments were carried out on 11 rabbits, of which 2 were 3 kg in weight or more while the remaining were approximately 2 kg; the age of the animals was 5-6 months at the beginning of the experiment. Bilateral orchiectomies were carried out aseptically via the peritoneal cavity under ether narcosis. After definite intervals the rabbits were sacrificed by means of air emboli. The length of the trials ranged from 2 to 337 days. The accessory sex glands, in complex with the urethra, were fixed in 10% formalin and sectioned transversely into 5-6 segments. After being imbedded in celloidin-paraffin, histological sections were prepared from each segment. Staining was performed with hematoxylin-eosin and according to the method of van Gieson. Several sections were stained according to Mallory and impregnated with silver by the method of Foote. In a number of cases a graduated series of sections were stained.

RESULTS

No deviations from the normal structure of the urethra were noted. Rarely, small groups of leucocytes were encountered in the coils of the lumen, but there were no leucocytes in the epithelium or the connective tissue.

In the ductus deferens the width of the lumen and the height of the epithelium had decreased. Disintegrating cells, in the form of pale shadows, were encountered in the lumen of the ampulla in 3 cases (at durations 27, 34, and 337 days); among them were small numbers of spermatozoa and isolated macrophages. In addition, phagocytosis of bits of cellular breakdown and sperms were noted in the protoplasm of the macrophages.

The seminal vesicle and its glandular crypts diminished, and the epithelial papillae in the crypts became smoothed. Epithelial secretion ceased, and the height of its cells was reduced. Transitional epithelium was also found in the seminal vesicle in 7 cases, but in the distal portions of the vesicle it was observed under normal conditions as well. No marked alterations appeared in the connective tissue of the vesicle; only in one case (experimental duration 115 days) was there observed a focal inflammation in the loose connective tissue between the seminal vesicles and the ducti deferentia. The lymphatic vessels in the area were dilated, and the connective tissue edematous; focal accumulations of macrophages, plasma cells, and lymphocytes, with admixed leucocytes containing segmented nuclei, were noted in the vessels and, here and there, in the connective tissue as well.

The utricular gland, even in the first days of the experiment, decreased in dimensions. As a result of the contraction of the glandular cavities, the double-row arrangement of the epithelium was more clearly defined, and, in places, the epithelial cells were arranged in 3 to 4 rows. No secretory granules were observed. In several of the cavities and in the ducts, homogeneous, pale staining fluid was noted in small quantities, and, occasionally, groups of macrophages containing foamy protoplasm. Over the course of the first 15 days of the experiment, intraepithelial cysts were encountered in all cases, arising as a result of the degeneration of several cells in a stratum of the epithelial layer. At later intervals no cysts were detected. At 2 months the majority of cavities collapsed into contact with the opposing wall; along with this the glandular parenchyma acquired the appearance of solid epithelial complexes, in which were preserved small remnants of the lumen. The epithelial cells, at this time, had decreased in dimensions, mainly at the expense of the protoplasm. As a result of the

Fig. 1. Collapse of the prostate gland. Atrophy of the epithelium. Stained by the method of van Gieson. Experimental interval, 55 days. Microphotograph. Magnification 70x.

Fig. 2. Collapse of the terminal divisions and marked dilatation of the major ducts in the Superior Cooper's gland. Stained by the method of van Gieson. Experimental interval, 80 days. Microphotograph. Magnification, 40x.

collapse of the glandular cavities, the amount of connective and muscle tissue was found to have increased correspondingly. Argyrophilic and collagen fibers condensed in the internal capsule, producing the impression of a degree of sclerosis. However, no actual proliferation of the connective tissue was noted.

Changes in the prostate gland were rather similar to the changes in the utricular gland (Fig. 1). In comparison with the latter, the collapse of the glandular cavities in the prostate occurred faster. Along with atrophy of the majority of the epithelial cells in the collapsed glandular alveoli, isolated hypertrophic cells were encountered.

The paraprostatic or Superior Cooper's glands were lacking in secretory material. Only in the distal divisions of the major ducts was a small amount of the palely staining homogeneous content encountered at both early and late intervals. The terminal divisions and the fine excretory ducts were collapsed at the end of the first month, and their lumina became indistinguishable. The epithelial cells decreased in volume, and their protoplasm became homogeneous. The major ducts remained wide or even dilated, often asymmetrically-only the left or the right (Fig.2). No proliferation of the connective tissue was noted.

In the bulbourethral or Inferior Cooper's gland, at the end of the first month, there occurred a collapse of the terminal divisions; many of them lost their lumen and appeared like continuous cellular masses. No signs of secretion were discovered in the epithelium, and the dimensions of its cells

were somewhat decreased. A scanty amount of the palely staining content was noted in the lumen of the major ducts in isolated cases.

SUMMARY

Castration in rabbits provokes atrophy of accessory sexual glands with complete closure of the glandular cavities and epithelial atrophy. There was no proliferation of connective tissue or inflammatory phenomena. Sperms may be retained in the ampulla of ductus deferens of castrated animals for prolonged periods of time (up to 337 days).

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GROWTH CHARACTERISTICS IN MAMMARY GLAND TISSUE CULTURES FROM MICE OF A NONCANCER LINEAGE (D) ASSOCIATED WITH THE ADDITION OF ANDROSTERONE AND ESTRADIOL 17$^{\beta}$ TO THE NUTRITIVE MEDIUM

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In the present work we have undertaken the problem of observing the behavior of mammary gland tissue in cultures in which female or male sex hormones have been added to the fluid medium.

A. A. Maximov [4], in 1925, first cultivated mammary gland tissue from rabbits with the addition of fragments of a yellow body; he did not obtain positive results, inasmuch as the yellow body degenerated very early. F. Elias [2] cultivated the mammary gland tissue of pregnant mice, and established, as we did [1], that the growth of epithelium is observed only when the experiment is performed within the first half of the pregnancy. With the addition to the cultures of estrone, progesterone, cortisone, growth hormone, and mammatropic hormone, the most prolonged epithelial growth was noted only in association with the simultaneous action of the growth hormone plus the mammatropic hormone. All the remaining hormones, both in combination and individually, were ineffective in this regard. E. Lasfargues [3], using the same subject with the addition of serum from the unbilical cords of humans, observed epithelial growth up to 30 days; with normal serum the cultivation duration was considerably less. V. Monesi [5], cultivating adenocarcinoma of the mammary gland of the mouse in the presence of estrone, progesterone, and testosterone, noted a stimulatory action on the part of estrone and progesterone, and a strongly depressant effect from testosterone.

METHOD

In the experiments we used 40 mice (20 virgins and 20 parous) of noncancer lineage (D), from 2 to 6 months of age. In the first series of experiments we introduced androsterone into the fluid nutritive medium, while in the second we used estradiol 17$^{\beta}$. For the seeding we took thoracic and abdominoinguinal mammary glands, divided them up, and seeded them in 6 flacons, 4 pieces in each; 3 of the flacons served as the control. The nutritive medium consisted of a mixture of heparinated chicken plasma, Henk's solution, an extract of 10-day-old chicken embryos, and group IV (AB) human serum. In the flacons with the experimental cultures we added the hormones, diluted in six drops of nutritive medium: estradiol 17$^{\beta}$—0.05γ and androsterone—0.1γ. The fluid nutritive medium was replaced after 4-5 days; along with this we added the hormone to the flacons each time in the same quantity and dilution. The cultures were studied from the moment of seeding up to 45 days—alive and in the fixed and strained preparations. Fixation was carried out with 10% formalin, Zenker-formol, and Carnu's solution. A portion of the culture was imbedded in celloidin for serial sections. The total cultures and sections were stained with Karatstsa's hematoxylin, iron hematoxylin by the method of Yasvoin, azure eosin, Mallory's, van Gieson's, and Sudan III.

RESULTS

Mammary Gland Cultures from the Virginal Mice. In the control series we seeded 240 fragments from 20 animals. Growth of the epithelium was obtained only in 2 cultures from one animal, while growth of connective tissues was observed in 212 cultures from 19 mice. The epithelium began to grow on the 4th day. Its membranes did not attain large proportions; they had a multilayered structure, which, toward the periphery, thinned out and in the border zone became unilayered. The cells were uniform elongated forms, and the oval nuclei at the periphery of the membrane were coarse. Despite the fact that mitoses and amitoses were observed in the epithelium in the first days of growth, the dimensions of the membranes increased by only a small degree. Dystrophic changes were already noted on the 13th day of explantation. Growth of the connective tissue occurred on the 2nd day. At the end of the 4th day the growth zone surrounded the fragment from all sides; numerous mitoses were observed in it. The nuclei of the fibroblasts differed little from each other in form

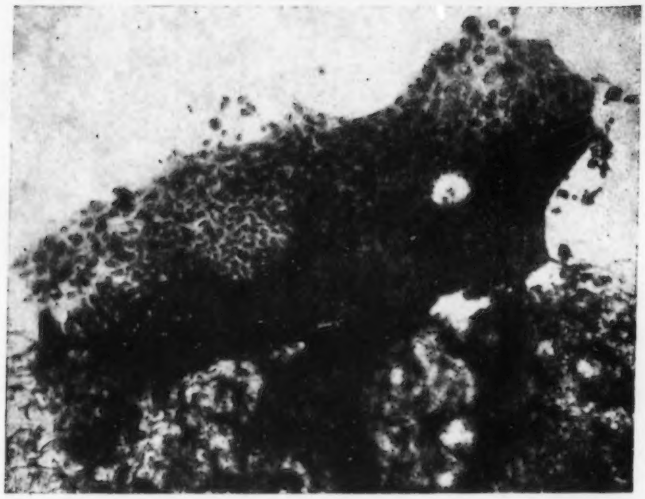

Fig. 1. Twelve-day old total culture of mammary gland epithelium, from virginal mice of a noncancer lineage (D), with androsterone added. Over-all view of the growth zone. Two replacements of the fluid phase. Karatstsa's hematoxylin. Magnification: ocular 7×, object. 8×.

and size. We were able to cultivate the connective tissue for 25 days.

One hundred and twenty fragments from 10 mice were seeded with the addition of androsterone. Epithelial growth was observed in 9 cultures from 4 animals, while connective tissue growth was obtained in 103 cultures from all the animals. The epithelium began to grow on the 2nd day. Initially, there appeared multilayered membranes and projections from the uniform cells, which, subsequently, expanded into a unilayered membrane of closely joined cells, forming an even border at the periphery (Fig. 1). Mitoses were noted in the growth zone from the first days of cultivation. The majority of the cells had an elongated form and an oval nucleus. Beginning with the 6th day amitoses were encountered as well as mitoses; there appeared polymorphic cells, differing considerably from one another in their dimensions. Their nuclei also differed in form and size and contained from 1 to 6 coarse nucleoli. Fine fat droplets were noted in the cytoplasm, beginning with the 7th day; uniting, they formed a single coarse droplet which deformed the nucleus. By the 12th to 14th day the number of mitoses decreased, dystrophic changes gradually developed, and, despite replacement of the fluid nutritive medium and trimming, the epithelium perished. The duration of cultivation was 17 days.

Just as in the case of the control, we were able to cultivate the connective tissue for a longer period of time than we could the epithelium. The effect of androsterone on the tissue was manifested by the appearance of a large number of bi- and trinucleated cells.

One hundred and twenty fragements from 10 animals were seeded with the addition of estradiol 17^{β}. Epithelial growth was obtained in 12 cultures from 4 animals, while connective tissue growth was noted in 102 cultures from all the animals. The epithelial growth began on the 2nd day, and on the 8th to 10th day the membranes had attained larger proportions than in the series with androsterone. The intercellular spaces were considerably wider, and, thus, the membranes had a looser structure (Fig. 2). The border of the growth zone was always irregular, and bore projections of various forms and lengths; the cells here were flattened, and they became coarser and adopted a diverse form. Polymorphism of the cells and nuclei, and differences in their size, were very markedly manifested. If in the series of experiments using androsterone the smallest cell differed from the largest by a factor of 3, then in the experiments with estradiol this factor was equal to 6-7. An analogous difference was also noted in the dimensions of the nuclei. In this series the epithelial growth was more intensive than in the series of experiments using androsterone; mitoses and amitoses were more frequently observed, and the growth zone was wider. As usual, fat appeared in the cytoplasm on the 6th-7th day. Dystrophic changes occurred in several cells on the 16th-18th day; we were able to carry a portion of the culture for 23 days.

Growth of the connective tissue was accompanied by the appearance of bi-, tri-, and pentanucleated fibroblasts. It was possible to cultivate the connective tissue for 35 days.

Mammary Gland Cultures from the Parous Mice. In the control series we seeded 240 fragments from 20 mice. Epithelial growth was noted in 11 cultures from 4 animals, while connective tissue growth occurred in 180 cultures from all the mice. The epithelium began to grow on the

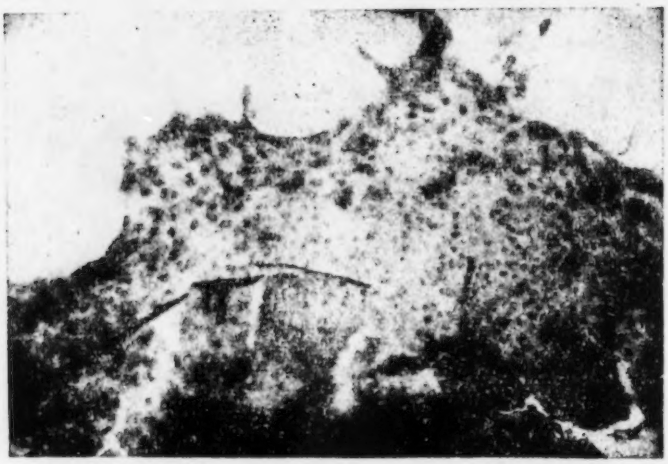

Fig. 2. Ten-day old total culture of mammary gland epithelium, from virginal mice of a noncancer lineage (D), with estradiol 17^{β} ad-added. Over-all view of the growth zone. Two replacements of the fluid phase. Karatstsa's hematoxylin. Magnification: ocular 7×, object. 40×.

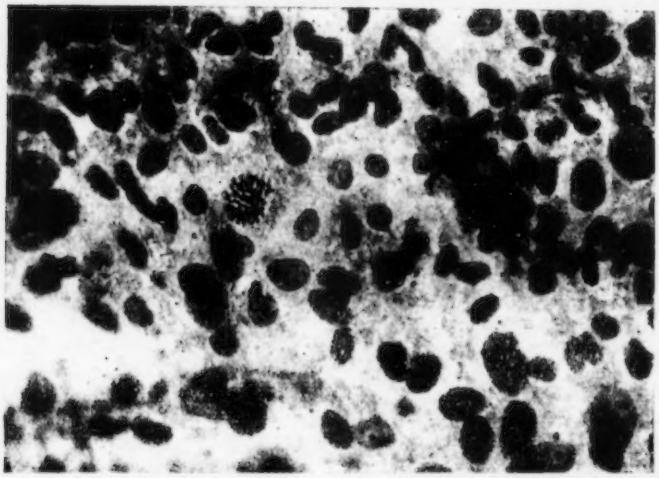

Fig. 3. Nine-day old total culture of mammary gland epithelium, from parous mice of lineage (D), with estradiol 17^{β} added. Nuclear polymorphism. Mitoses. Two replacements of the fluid phase. Glandular hematoxylin. Magnification: ocular 7×, object. 40×.

3rd-4th day; multilayered and unilayered membranes formed on the 5th to 6th day. Mitoses were encountered rather rarely in them. The form and size of the cells and nuclei underwent insignificant variation. The epithelial growth zone did not attain large proportions. Fat appeared in several cells on the 6th to 7th day. Growth and multiplication of the epithelium was observed up until the 16th to 18th day. On the 20th to 25th day the epithelium perished. Connective tissue growth was more rapid than epithelial growth; mitoses were encountered in it rather frequently. The cells were fundamentally similar to one another, and had a fusiform shape. The connective tissue grew for 30 days.

One hundred and twenty fragments from 10 animals were seeded with the addition of androsterone. Epithelial growth was obtained in 5 cultures from 3 animals, while connective tissue growth was noted in 94 cultures from all the animals. Growth of both tissues began on the 2nd day. The epithelium formed membranes and projections with densely distributed cells. Growth intensity was greater than

Data on the Cultivation of Mammary Gland Tissue from Mice of a Noncancer Lineage (D)

Group of mice	Control			Androsterone			Control			Estradiol 17β		
	seeded	growth obtained		seeded	growth obtained		seeded	growth obtained		seeded	growth obtained	
		epitheli-um	connec-tive tissue		epitheli-um	connec-tive tissue		epitheli-um	connec-tive tissue		epitheli-um	connec-tive tissue
	A/B	B/C	B/C	A/B	B/C	B/C	A/B	B/C	B/C	A/B	B/C	B/C
Virginal	120/10	2/1	105/9	120/10	9/4	103/10	120/10	—	107/10	120/10	12/4	102/10
Parous	120/10	6/2	88/10	120/10	5/3	94/10	120/10	5/2	92/10	120/10	7/3	99/10

Notes: 1. Cultures with simultaneous growth of epithelium and connective tissue were classified in the group of cultures with epithelial growth. 2. A: number of fragments; B: number of animals; C: number of cultures.

in the cultures of the control series, and the corresponding growth was wider. Mitoses were encountered constantly, from 1 to 2 per visual field; amitoses were also observed along with them. Nuclear and cellular polymorphism was markedly manifested only in spots; the nuclei sometimes attained very large proportions. The majority of nuclei had an oval form: they contained up to 8 coarse nucleoli,, varying in shape and size. Cells with 2-4 nuclei appeared in the growth zone on the 8th to 10th day. Beginning with the 7th day fat appeared in the cytoplasm, initially in the form of droplets which united into a singly coarse drop and deformed the nucleus. We were able to cultivate the epithelium for up to 20 days.

The connective tissue grew more actively than in the control. Many mitoses and amitoses were observed in it, and also cells, in large number, containing from 2 to 6 nuclei. The duration of cultivation was 40 days.

One hundred and twenty fragments from 10 animals were seeded with the addition of estradiol 17$^{\beta}$. Epithelial growth was observed in 7 cultures from 3 animals, while connective tissue growth was noted in 99 cultures from all the mice. The epithelium and connective tissue, as in the other series of experiments, began to grow on the 2nd day. A peculiarity of the series of experiments with estradiol appeared to be the presence of epithelial membranes with loosely distributed cellular elements. Polymorphism of the cells and nuclei and variation in their dimensions were manifested very markedly (Fig. 3). Almost in every culture cells were encountered with 3-4-6 nuclei or with one very coarse nucleus. The nucleoli, numbering from 1 up to 10, were coarse and variable in form. Sometimes rod-shaped nucleoli seemed to divide the nucleus into pieces. In cultures of this series a very large number of mitoses were observed; in a single visual field it was possible to see up to five. Often 2-4 polar mitoses were encountered. Sometimes the width and the structural characteristics of the membranes in the same culture were dissimilar. Along with loosely structured, unilayered portions there were also dense, multilayered parts. The dimensions of the cells and nuclei in the latter were considerably smaller than in the loose membranes, the polymorphism weakly manifested, and the multinucleated cells encountered as an exception. Mitoses and amitoses were observed more rarely. Multipolar mitoses were absent. Beginning with the 6th day fat appeared, as usual, in the cytoplasm of several of the cells.

Degenerative changes in this series occurred later. We were able to cultivate the epithelium for up to 25 days.

The connective tissue grew more energetically than with the addition of androsterone. Mitoses and multinucleated cells also were more numerous. The duration of cultivation was 45 days.

The results of our investigations are presented in the table. If one is orientated by the percent of growing epithelial cultures in relation to the number of fragments of mammary gland tissue seeded and the number of animals from which fragments were taken, i.e. by the output of

cultures, then the effect of the action of androsterone and estradiol 17^{β} emerges with sufficient conclusiveness only in the group of virginal mice. In this group in two control series (total number of mice–20, from which 240 fragments were seeded) only 2 cultures from one animal grew out. At the same time, in the series of experiments with androsterone (10 mice, 120 fragments) 9 cultures from 4 mice grew out, and in the series with estradiol 17^{β}, 12 cultures from 4 of the 10 animals successfully grew.

In the group of parous mice the addition of androsterone and estradiol 17^{β} did not show any effect on the number of growing cultures as compared with the control. Along with this, the addition of androsterone and estradiol 17^{β} reflected itself in principally the same manner on the intensity of growth and culture morphology of both the virginal and parous mice, the only difference being that the changes were manifested considerably more clearly in the experiment with estradiol 17^{β}. The action of both hormones, in comparison with the control, made itself apparent in the quantitative increase in mitoses, the multipolarity within them (possible also amitoses), in the formation of multinucleated cells, in the polymorphism of the cells and nuclei, and in the characteristics of the membrane: it was significantly wider in the series of experiments with estradiol 17^{β} than in the control or in the series with androsterone.

As far as the connective tissue from the mammary gland was concerned, the number of growing cultures was similar in both the control and the experimental series, and in both the virginal group and the parous group of animals. However, here the action of androsterone and estradiol 17^{β} was expressed in the appearance of a large number of multinucleated cells, especially in the experiments with estradiol 17^{β}.

Thus, the addition of androsterone or estradiol 17^{β} to cultures of mammary gland tissue bears evidence in favor of the feasibility of their direct morphogenetic activity on the epithelium and connective tissue of the mammary gland. This action is apparent in both a fortification of the process of mitotic division and in its disruption, which is attested by the presence of pathological mitoses and multinucleated cells in the cultures. In this regard, estradiol 17^{β} is more active than androsterone.

SUMMARY

The author cultured the tissues of the mammary gland obtained from 40 mice (aged from 2 to 6 months) of non-cancer lineage (D). Androsterone was introduced into the fluid nutritive medium in the first series of experiments, and estradiol 17^{β} – in the second. The effect of the action of androsterone and estradiol 17^{β} was sufficiently convincing only when culturing the tissues of the virgin mice (according to the number of the growing epithelial cultures). The effect of androsterone and estradiol on the growth intensity and the morphology of the epithelial cultures was reflected in a principally similar way both in the virgin and parous mice, the only difference being that these changes were more clear-cut in the experiment with estradiol 17^{β}. The effect of the hormones was manifest in the increased number of mitoses, including multipolar (possible also of amitoses), in the formation of the multinuclear cells, the polymorphism of the cells and nuclei, the greater width of the membrane in the series of experiments with estradiol 17^{β} (in comparison with control and the series of experiments with androsterone). The number of the growing connective tissue cultures of the mammary gland were the same both in the control and in the experimental series, in the group of virgin and in the group of parous animals. Here the effect of estradiol 17^{β} and androsterone was manifested in the appearance of a large number of multinuclear cells, especially in the experiment with estradiol 17^{β}.

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THE CHARACTER OF THE 24-HOUR PERIODICITY OF MITOSIS IN THE CORNEAL EPITHELIUM OF VARIOUS LABORATORY ANIMALS

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In the last few years the attention of many investigators has been devoted toward studying the role of factors of the external environment in the phenomena involved with the 24-hour rhythm of cell division processes that take place in the tissues of an organism [2, 5, 6, 7, 9, 10].

It has been established that 24-hour periodicity of mitotic activity is characteristic of the majority of tissues in the organism. Despite the fact that the question of 24-hour periodicity of mitotic activity has been dealt with in a considerable number of works, up until now there has not been performed a comparison of the 24-hour rhythm of mitosis in the same tissue of different species of animals. Along with this, a comparative approach to the study of this phenomenon would be able to aid the understanding of the mechanism of cell multiplication and the conditions that provide for one or another intensity in the proliferation of cellular elements.

The purpose of this investigation was to study the 24-hour periodicity of mitosis in the corneal epithelium of animals of different species at different times of the year.

METHOD

The investigation was carried out on male white mice (18-20 g in weight), white rats (130-150 g in weight), and guinea pigs (300-350 g in weight). The animals were sacrificed in the course of a 24-hour period at 6-hour time intervals. The larger portion of the experiments were begun at 8:00 in the evening, while several of the experiments were started at 8:00 in the morning, the purpose of this being to test the influence of the time of the experiment's beginning on the character of the 24-hour rhythm of mitotic activity observed.

The experiments were performed in the various seasons of the year: spring (in March), summer (in June and August), fall (in September) and winter (in December and January).

Before setting up the experiments the animals were kept under standardized conditions for 6-7 days. All 24-hour experiments were carried out in periods of maximum quiet within the laboratory rooms (on Saturday evening and Sunday). In order to reduce to a minimum the effect of various secondary external factors on the 24-hour regime of mitosis, we fed the animals and took samples of the cells at the same time in the 24-hour period. In addition, sacrifice of the animals was carried out in a neighboring room, and not in the experimental chamber.

Following fixation of the cornea in Carnu's solution and subsequent staining with hematoxylin by the method of Karachcha, total preparations were made from it. For the determination of mitotic activity we investigated the entire epithelium of the cornea and compiled a chart of the distribution of mitoses, recording the stage of cell division. For the mitotic index we used the number of mitoses per 100 visual fields, which, using the diaphragm we selected, corresponded to 1 mm^2 in corneal area. The index was calculated the same way, whether for the entire cornea as a whole, or for isolated zones within it (peripheral and central).

Eighteen series of experiments were set up, on 360 animals. At each interval of the investigation 5 animals were sampled.

RESULTS

The data on the mitotic regime of the corned epithelium, during the course of 24 hours, in mice, rats, and guinea pigs, are presented in the figure. The trial described was carried out in August.

As is apparent from the data presented, 24-hour periodicity of the mitosis in the corneal epithelium of mice was clearly manifested in the form of a monophasic curve. The smallest number of cell divisions in the corneal epithelium of the white mice was observed in the evening hours. The mean mitotic index at that time of the 24-hour period was, on the average, 45 mitoses per 100 visual fields.

At 2:00 A.M. the number of cell divisions in the investigated tissue clearly increased (198 mitoses) and attained a maximum in the morning hours (437 mitoses).

A marked fall in the mitotic activity of the corneal epithelium was already observed at 2:00 P.M. (140 mitoses).

The curve of the changes in mitotic activity of the corneal epithelium in white rats over the course of 24 hours (see figure) repeated the tracing of the 24-hour curve for the white mice. However, in the corneal epithelium of the white rats the range of the 24-hour fluctuation in the num-

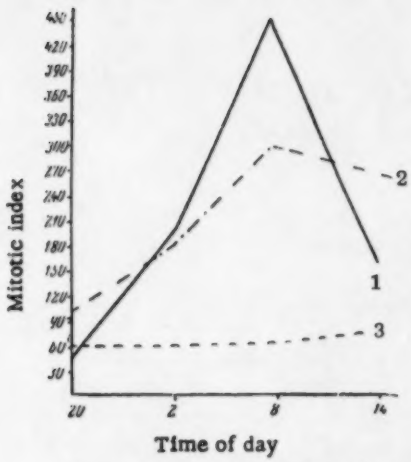

Figure. The 24-hour rhythm of mitosis in mice (1), rats (2), and guinea pigs (3).

ber of dividing cells was demonstrated to a lesser degree.

In the corneal epithelium of the white rats, as in the case of the white mice, the number of mitoses in the evening hours was always minimal; on the average it was equal to 95 mitoses. In the night hours the number of dividing cells increased; the mean mitotic index at that time reached 179.

The maximum number of cell divisions in the corneal epithelium of the white rats was noted in the morning hours (297 mitoses). In the afternoon hours (2:00 P. M.), as a rule there occurred reduction in the total number of dividing cells of the corneal epithelium; the mean mitotic index was lowered to 260.

In contrast with the data obtained from the white mice and white rats, no 24-hour rhythm of mitosis was observed in the corneal epithelium of the guinea pigs. The magnitude of the mean mitotic indices in their case did not essentially change in the course of the 24-hour period. The mitotic index throughout the full 24 hours ranged within the borders of 63-76 mitoses (see figure).

The results of the experiments on the study of the 24-hour rhythm of mitotic activity in the corneal epithelium of white mice, white rats, and guinea pigs during the various seasons of the year are presented in the table.

The character of the 24-hour changes in mitotic activity in the experimental animals was maintained as approximately the same in all seasons of the year.

In the mice and rats the maximum number of mitoses were always observed in the morning hours, and the minimun, in the evening. The reliability of the discrepancy between the maximum and minimum indices was sufficiently high in all cases.

As far as the mitotic activity of the corneal epithelium in the guinea pigs is concerned, regular shifts in the 24-hour curve were not observed, as a rule, during the various seasons of the year. However, in the summertime (June) a certain increase was detected in the number of dividing cells during the evening hours as compared with the remaining intervals of the investigation. Statistic treatment of the material showed that this difference was close to being statistically significant ($P = 0.03$).

The distribution of the dividing cells in the separate zones of the corneal epithelium at different hours of the day was not the same. The increase in the mean mitotic index of the corneal epithelium in the white mice during evening time occurred as a result of the increase in the number of dividing cells in the peripheral zone of the tissue. At 2:00 A. M. there was an average of 3-5 times more mitoses in the periphery of the cornea than in the central zone. The reduction in the over-all mitotic index for the corneal epithelium of the white mice during the daytime hours of the 24-hour period also occurred primarily at the expense of the peripheral zone. The number of cell divisions in the perip-

The Mitotic Activity of the Corneal Epithelium in the Course of 24 hours During the Various Seasons of the Year[1]

Species of animal	Time of day \ Months	January	March	June	August	September	December
Mice	8:00 P.M.	45±4	22±2	39±11	45±19	21±5	55±9
	2:00 A.M.	194±42	221±31	210±39	198±38	247±39	199±45
	8:00 A.M.	347±25	448±23	438±48	437±22	392±25	301±28
	2:00 P.M.	121±18	152±11	169±21	140±21	129±19	72±20
Rats	8:00 P.M.	153±32	76±18	185±11	95±13	45±18	90±32
	2:00 A.M.	279±20	171±39	181±26	179±16	182±29	241±19
	8:00 A.M.	272±26	362±25	354±21	297±10	261±62	311±22
	2:00 P.M.	272±20	191±21	217±17	260±16	340±22	207±27
Guinea pigs	8:00 P.M.	85±6	96±18	87±7	63±7	69±5	75±5
	2:00 A.M.	109±26	81±1	71±9	63±2	82±5	69±6
	8:00 A.M.	108±17	85±8	100±4	67±8	84±5	78±9
	2:00 P.M.	111±8	84±6	85±6	76±7	100±7	82±6

[1]The mean values of the mitotic indices are presented in the table.

hery of the cornea at 2:00 P. M. was, on the average, 1½ times smaller than in the central zone.

Summing up the material presented above, it can be noted that there exist species-specific differences in the mitotic regime of the corneal epithelium of laboratory animals.

They are concerned with both the intensity of proliferation and the character of the 24-hour dynamics of mitotic activity. The mean 24-hour mitotic index for the corneal epithelium of white mice and rats was higher than in guinea pigs.

The most clearly demonstrated 24-hour periodicity of mitosis appeared in the mice.

In mice the number of mitoses in the morning was greater than in the evening by an average of 12 times, while in rats it was greater by only 3 times. Our data on the 24-hour periodicity of mitosis in the corneal epithelium of white rats corresponded to the data of M. T. Gololobova [1].

No regular shifts in the 24-hour rhythm of mitosis were observed in the corneal epithelium of the guinea pigs. In connection with this, it should be noted that the dynamics of the motor activity of guinea pigs are characterized by the absence of a regular 24-hour rhythm [7].

Guinea pigs differ from mice and rats in that they manifest a less dynamic mitotic activity in their corneal epithelium [2, 3].

SUMMARY

The 24-hour periodicity of mitosis was studied in the corneal epithelium of white mice, white rats and guinea pigs. There were species-specific differences both in the intensity of the cellular multiplication, and in its 24-hour rhythm. The 24-hour periodicity of mitotic activity is clearly manifested in mice and rats, the maximal amount of mitoses being observed during morning hours, and the minimal—in the evening. The curve, characterizing the 24-hour periodicity of mitoses is uniform during all the seasons of the year. The amplitude of the 24-hour variation of mitotic indices in mice is on the average 3 to 4 times greater than in rats. In guinea pigs the 24-hour mitotic periodicity of the corneal epithelium is not manifested so clearly.

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THE ELECTROCARDIOGRAM OF HEALTHY RABBITS IN STANDARD AND CHEST LEADS AND METHODS OF RECORDING IT

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In spite of the fact that rabbits are widely used in experimental studies of the cardiovascular system, we have found no description in the accessible Soviet literature of the normal electrocardiogram in standard and chest leads or of methods of recording it.

In a few papers published abroad, various accounts are given of fairly laborious methods of recording the electrocardiogram in standard and chest leads, and also of different variants of the normal electrocardiogram. We found no description of the normal electrocardiogram in four chest leads. Some authors recorded the electrocardiogram with the animal in the normal position [8, 13], others with the animal lying in the supine position [6, 9]. Different methods are described in individual papers: introduction of the electrode along with a tube into the esophagus (under x-ray control) [16], the application of plates or of coils to the epilated skin of three of four limbs, soaked in various solutions [4, 5] or to the surface of the chest on the right and left sides [8]; Slapak and Hermanek [15] and also other authors used injection needles as electrodes. Lepeschkin [7] mentions the bipolar chest lead, the so-called axial of Schinzel [14]. In this lead, one electrode is fixed to the right shoulder and the other in the region of the apex of the heart, corresponding to the anatomical axis. The recording is made in the usual way, or by Wilson's method.

In leads from the limbs, the electrocardiogram of the rabbit is usually of low voltage and the waves are not always clearly shown. In the chest leads the changes in the electrocardiogram appear sooner, last longer, are clearer and enable the potential of a given point of the heart to be determined.

The absence of details of the normal electrocardiogram of rabbits and of a single, simple method of recording it, led us to describe our own observations on this problem.

METHOD

Electrocardiograms were recorded in 36 young and healthy rabbits every 2-2$^1/_2$ months: in one group of 18 animals, for a period of one year, and in the other group for 20 months. In each rabbit the electrocardiograms were taken from 5 to 8-9 times; altogether 220 electrocardiograms were taken in the standard and the four chest leads. From 7-10 minutes before the electrocardiogram was recorded, the animals were secured to the bench lying on their back. For electrodes we used thin steel needles, which (after treatment with alcohol) were injected subcutaneously in the dorsal aspect of the distal segments of three limbs (2 forelimbs and the left hindlimb) for recording the standard leads. The electrocardiograms were recorded on an ÉKP-4m apparatus, with a sensitivity of 1 mv = 2 cm and a film movement velocity of 7-8 cm/sec. As in man, when we recorded the electrocardiogram in the chest leads, we took

Fig. 1. Position of the rabbit and points on the chest wall from which the electrocardiogram is recorded.

TABLE 1. Heart Rate, Duration of Waves, Magnitude of Electrical Systole of Ventricles and Arrangement of Electrical Axis of the Heart in Healthy Rabbits

Rabbit No.	Type of electrocardiogram	Range of variations, heart rate/min	Mean Heart rate per min	Duration of waves and intervals (in hundredths of a second)			Magnitude of electrical systole of ventricles Q–T (in hundredths of a second)
				P	*PQ*	*QRS*	
31	Mixed	330—300	300	0,03	0,06	0,04	0,14
51	Left	300—280	290	0,03	0,06	0,04	0,13
10	Vertical	300—280	290	0,04	0,07	0,04	0,14
12	Right	240—300	285	0,04	0,06—0,07	0,03—0,04	0,14—0,15
17	Left	280—220	250	0,04	0,06	0,04	0,15
19	Vertical	300	300	0,03—0,04	0,06	0,03	0,14
19a	Normal	300—260	280	0,04	0,07	0,03	0,14
20	Vertical	300—280	290	0,04	0,07	0,04	0,14
21	Left	280—260	270	0,04	0,07	0,04	0,15
23	Normal	260—320	300	0,03	0,06	0,03	0,14
24	Mixed	260—310	300	0,03	0,06	0,03	0,14—0,15
25	Right	230—240	240	0,03—0,04	0,07	0,03—0,04	0,15
26	Left	300—330	310	0,03	0,06	0,03	0,14
27	Vertical	300	300	0,04	0,06—0,07	0,03	0,15
28	»	240—330	300	0,03	0,06	0,03	0,13—0,15
28a	Normal	300—300	300	0,04	0,08	0,03	0,13
29	Right	300	300	0,03	0,06	0,03	0,14
29a	Vertical	270—260	260	0,03	0,07	0,05	0,14
30	Right	300—320	310	0,03—0,04	0,06	0,03	0,13—0,14
32	Vertical	250—300	270	0,03—0,04	0,07	0,03	0,15—0,14
32a	Right	300—280	290	0,04	0,07	0,04	0,14
33	Mixed	240—320	280	0,03—0,04	0,06—0,07	0,04—0,03	0,15—0,14
33a	Right	300—280	290	0,04	0,07	0,04	0,14
34	Normal	240—280	260	0,03—0,04	0,07	0,03—0,04	0,15—0,14
35a	»	280—300	290	0,04	0,06—0,07	0,03	0,14
36	Right	240—280	270	0,03	0,07	0,03	0,15
37	Normal	260—320	300	0,03	0,06	0,03	0,14
37a	Right	300—280	290	0,04	0,07	0,04	0,14
39	Mixed	300—300	300	0,03	0,05—0,06	0,03	0,14
39a	Normal	300—260	280	0,03	0,07	0,03	0,15
42	»	260—180	220	0,04	0,08	0 03	0,15
44	Right	300—270	280	0,03	0,06	0,03	0,14
45	Left	310—320	315	0,03	0,07	0,03	0,13
46a	Right	270—300	290	0,03—0,04	0,06	0,03	0,13
47	»	300—300	300	0,03	0,07	0,03	0,13
49	»	300—240	270	0,04	0,07	0,05	0,15

into consideration the peculiarities of the animal. The needle electrode corresponding to the right limb was treated with alcohol and then inserted beneath the skin of the lateral part of the right forelimb to a depth of 1.5-2mm, and the electrode corresponding to the left limb was introduced at the following points of the chest wall (at the heart level): points I (CR) and IV (CR_5), at the place of intersection of a vertical line corresponding to the anterior axillary line with a horizontal line at the level of the apex of the heart; points II and III (CR_1 and CR_4) at the intersection of the parasternal lines with the horizontal line, corresponding to the lower and lateral sides of the thoraco-abdominal angle (Fig. 1). In the selection of these points we were guided by the anatomical structure and topographical situation of the heart in the thorax [2].

RESULTS

Comparison of the electrocardiograms recorded with the rabbits supine with those taken with the animals in the normal position satisfied us, as it did Massman and Opitz [9], that no significant difference could be detected.

The rhythm and rate of the heart. The heart rate varied between 240 and 300 contractions per minute (mean 250). In one case a rate of 180, and in another, of 330 contractions per minute was observed (Table 1). We found no disturbances of the sinus rhythm nor other evidence of heterotopic sources of cardiac contraction.

Arrangement of the electrical axis of the heart. We observed five arrangements of the electrical axis. In the normal type (Fig. 2a) an R wave was present in all three leads (sometimes a small Q_I or Q_{III}). The right type (Fig. 2b) was characterized by absence (or very small size) of the R wave in lead I, by the presence of a well-marked S_I wave (often S_{II}) and also by the presence of a well-marked

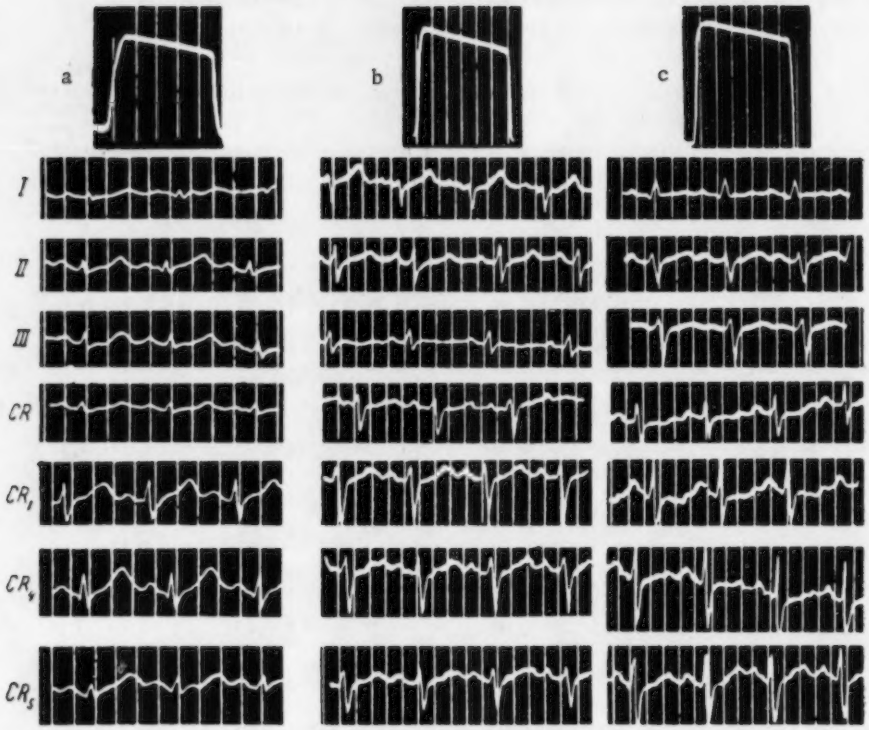

Fig. 2. Electrocardiogram of healthy rabbits in standard and chest leads. Variants of the arrangement of the electrical axis: a) normal; b) right; c) left.

R_{III} (sometimes a small R_{II}). Occasionally a deep Q_I was found instead of S_I, probably due to inversion of the entire QRST complex. Small Q_{III} and Q_{II} waves were often seen. In the left type (Fig. 2c) the R wave in lead I was quite well marked (often R_{II}). S_{III} was well defined. A small Q_I was often observed, and sometimes Q_{II}. The vertical type (Fig. 3a) was distinguished by the fact that in lead I the QRS complex was very low. In leads II and III the R waves were moderate in size and $QRS_I < QRS_{II} >$ or = QRS_{III} (sometimes Q_{III} was small). In the transitional type (Fig. 3b) it was difficult to determine the arrangement of the electrical axis for the signs of the various types were mixed.

According to our findings, the electrocardiograms in the chest leads usually correspond to the types in the leads from the limbs. The principal feature of the normal and left types was the presence of an R wave in all the chest leads, which increased as the electrode moved in the direction toward the left position III (CR_4), but at the point CR_5 it was often low. The S wave was well defined in CR, CR_I and CR_4; depending on the direction of the electrical axis, however, this wave was present to a different degree in positions I and IV, and might even be absent. In the normal type in the right chest leads r < S or r=s, and in the left R > s or r=s (see Fig. 2a). The R wave was diminished in the vertical type in the left leads. In the left vertical and normal types, the S wave in leads III and IV (CR_4 and CR_5) was usually ill-defined and no higher than the R wave (see Fig. 2a, b, c), but in the right type it was well marked and often greater than the R wave. Lepeschkin [7] points out that a direct lead from the right ventricle gives rS, and one from the left Rs. In chest leads taken on the left, on the chest wall at the heart level, corresponding to the point V in man, Levin [8] found an S wave in half these tracings.

In 18 rabbits, i.e., in half the total number, a right (in 10 animals) and a vertical (in 8 animals) type of arrangement of the electrical axis of the heart was observed. Predominance of the right type of electrocardiogram in healthy rabbits is also reported by L. P. Peresad'ko [1] and Massman and Opitz [9]. The two last authors determined that the electrocardiogram was mainly of the right type according to Schlomka's index. Mayeda [10] found a right type of electrocardiogram in newborn rabbits under ten days old. Agduhr and Stenström [4] observed left and right types of electrocardiogram. Slapak and Hermanek [15] described three types of position of the axis – left, vertical and normal. Within the limits of these types they observed many variants of deviation. Like Lepeschkin [7] and Slapak and Hermanek [15], we also observed spontaneous changes in the type of electrocardiogram in the same rabbit. After a short time the normal type became vertical, the vertical

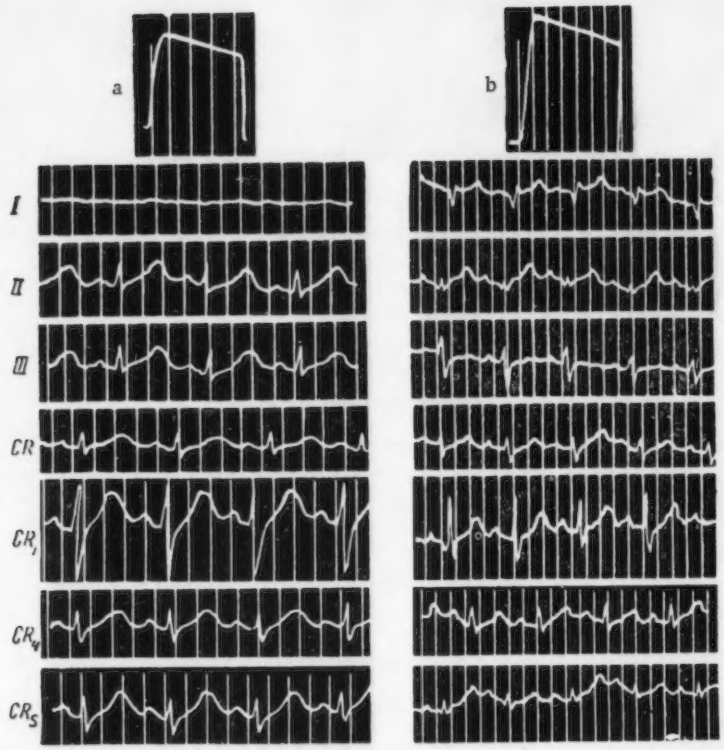

Fig. 3. Electrocardiogram of healthy rabbits in standard and chest leads. Variants of the arrangement of the electric axis: a) vertical; b) mixed.

became right, and so on. In conformity with the type, changes occurred in the relationship between the R/S waves in leads I-III and in the chest leads. These spontaneous changes, however, were mainly in connection with the transition between closely related types: for example, a normal into a left type, a vertical into a right type, and so on. A transition in a diametrically opposite direction, for example from right to left or vice versa, was never seen. Furthermore the new type of arrangement of the axis did not stay a long time fixed in its new variant, and often reverted to its original pattern. This account shows that changes in the type of electrical axis are associated not with changes in the muscle or in the conducting system of the heart, but with displacement of the heart in connection with changes in the position of the body, the height of the diaphragm (by a full stomach) and so on. This is confirmed by Boden and Neukirch [5], who observed changes in the type of electrocardiogram of the isolated rabbit's heart in accordance with rotation of the heart.

Changes in the waves and intervals of the electrocardiogram. The P wave was more pronounced in lead II and less so in lead III. In lead I it often was low or even obliterated. Lepeschkin considers that the P wave in lead I may be low or negative, but Slapak and Hermanek always found a positive P wave in all three leads. The degree of expression of the P wave often depended on the type of arrangement of the electrical axis of the heart. In the left type the P wave was more pronounced in leads I and II, and in the right and vertical types, it was more pronounced in leads III and II. The duration of the P wave was 0.03-0.04 seconds and its height 0.1-0.15 mv. These values agree with those cited by other authors (Table 2). The P wave in the chest leads was always positive. Its height varied from 0.03 to 0.025 mv, and its width from 0.03 to 0.04 seconds. Lepeschkin points out that in a lead from the apex of the heart, the P wave is always positive, and from 0.1 to 0.15 mv in height. Like Slapak and Hermanek and other authors, we were unable to find any obvious connection between the magnitude of the P waves and the arrangement of the electrical axis of the heart.

The Q wave was usually present in the left type in lead I and more rarely in lead II, and in the right type, in lead III and more rarely in lead II. The height of the Q wave usually did not exceed 0.1 mv. In one case, in lead I in the right and mixed types, instead of an S wave we found a Q, reaching a height of 0.4 mv. In the left, and also in the normal type of electrocardiogram, in the left chest leads and in CR_4 and CR_5, small Q waves (from 0.02 to 0.15 mv) were observed.

The P-Q (P-R) interval varied depending on the heart rate between limits of 0.07 and 0.08 seconds. Our values are close to the figures cited by Slapak and Hermanek, and

TABLE 2. Data of Various Authors on the Heart Rate, the Duration of the P Wave, P-Q Interval, QRS Complex and Ventricular Systole QT, and the Height of the P, R, S and T Waves

Authors	Range of variations in heart rate per minute	Mean heart rate per min	Duration (in hundredths of a second)				Height of waves in limb leads (in hundredths of a millivolt)									
							P			R			S		T	
			P	PQ	QRS	QT	minimum	maximum	mean	minimum	maximum	mean	minimum	maximum	minimum	maximum
Lepeschkin (Mayeda's figures)	150—365	250	0,03—0,04	0,05—0,10	0,015—0,04	0,12 at a rate of 250 contractions per minute	0,04	0,20	0,10	0	0,80	0,35	0	0,40	0,05	0,44
Slapak and Hermanek	187—352	200—300	0,03—0,06	0,05—0,07	0,02—0,03	0,11—0,15	0,10	0,20	0,15	0,03	0,80	—	—	—	0,02	0,40
Massman and Opitz	100—240	200	0,035—0,045	0,07—0,075	0,035—0,05	—	—	—	—	—	—	—	—	—	—	—
Ahdurh and Stenström	150—350	250	0,03—0,04	0,05—0,08	0,0175—0,03	0,10—0,15	—	—	—	—	—	—	—	—	—	—
Levin	174—282	225	—	0,06—0,09 occasionally 0.1 second	0,03—0,04	—	—	—	—	—	—	—	—	—	—	—
A. O. Saitanov	180—330	250	0,03—0,04	0,07—0,08	0,03—0,04 0.05 in two cases	0,13—0,15	0,03	0,25	0,13	0,02	0,70	0,40	0,02	0,50	0,02	0,5

are almost in agreement with those of Massman and Opitz (see Table 2). The P-Q interval was usually well defined in all leads and was disposed isoelectrically.

The QRS complex had a mean duration of 0.03-0.04 second (in two cases it was 0.05 second). Our values are close to those cited by Lepeschkin, and agree with those of Levin (see Table 2). The height of the QRS waves was different in each lead. The ratio between the R/S waves was mainly dependent on the direction of the electrical axis of the heart. The greatest height of the wave in the limb leads was 0.70 mv. Lepeschkin, and Slapak and Hermanek observed waves with a height of 0.80 mv (see Table 2). In the chest leads the duration of the waves was within limits of 0.03-0.05 second, and the height of the tallest wave was 0.9 mv. The depth of the waves reached 0.8 mv. According to Slapak and Hermanek the duration of the R wave in all cases was 0.03 second and the maximum height of the wave 0.9 mv (varying between 0.3 and 0.9 mv). In two healthy rabbits we observed for a short time a difference in the height of the R waves in one of the leads, which, in our opinion, was not pathological. Lepeschkin also draws attention to the possibility of spontaneous inversion of the QRST complex in leads I, CF_2 and CF_3.

The S-T interval was usually situated on the isoelectric line and was in the form of a straight line or, more rarely, slightly inclined. Sometimes in lead I (in the left type) or in lead III (in the right type) it was slightly convex upwards, and changed into a slightly biphasic (±) or, much more rarely, into a negative T wave. At the same time, in certain (2-5) leads we did not observe depression of a straight or curved S-T interval below the isoelectric line by more than 0.05-0.1 mv. Slapak and Hermanek [15] and also Mayeda[10]observed depression of the T-S interval only in exceptional cases. According to the findings of Massman and Opitz, the S-T interval was always isoelectrical, and the T wave did not arise from the R wave. Levin found in certain cases the appearance of a T wave without an isoelectrical interval, directly from the R and S waves, and Mayeda observed this to occur from the R wave only. High elevation of the S-T interval in one of the three types of electrocardiogram of the rabbit was described by Büchner and Licadov [6].

In some cases we observed in the chest leads an inclined, depressed (by more than 0.1 mv) S-T interval, leading to a positive T wave. In chest lead I (CR) an S-T interval convex uppermost was often encountered, which passed into a low and slightly biphasic (∓) T wave. The constant depression of the straight or curved S-T interval by more than 0.05 0.1 mv in certain standard or chest leads must be regarded as a sign of a pathological state of the myocardium.

The T wave was always positive in lead II and in most cases in Lead I. In lead III, more often than in lead I, it was considerably modified—depressed, smoothed out or even inverted, depending on the disposition of the electrical axis of the heart. In the vertical and right types the T wave was more positive in leads I and II and, in the majority of cases, in lead III. In lead III, however, the T wave was often low and even inverted. In the left type the T wave was often positive in lead I, but more frequently than in the other type it was low or even biphasic (∓). Our results are in agreement with Mayeda's observations. Massman and Opitz point out that in their findings a negative T wave in one lead was found in 3% of healthy rabbits, a flattened T wave in 30%, but an isoelectric T wave in certain leads was found in only 4 of 81 rabbits. The height of the positive T waves in the limb leads varied between 0.02 and 0.5 mv (in the majority, the average value was 0.3 mv). In most cases, the T wave was positive in all the chest leads. In the right type of disposition of the electrical axis, in a few cases it was flattened or smoothed out in the left leads (CR_4 and CR_5). In the left type, in a few cases the T wave in chest lead I (CR) and in lead II (CR_1) was flattened, smoothed out or, more rarely, biphasic (-+).

Whatever the type, the T wave in positions III and IV (CR_4 and CR_5) was always positive (in the left type it was occasionally low). In the remaining cases a persistent fall in the T wave in some chest leads always indicated pathological changes in the heart muscle. The maximum height of the T wave reached 0.6 mv. According to Slapak and Hermanek the height of the T wave was between 0.1 and 0.3 mv.

The electrical ventricular systole (Q-T) depended on the heart rate and varied from 0.13 to 0.15 second (see Table 1). A similar relationship between the duration of ventricular systole and the heart rate is noted by Lepeschkin, Agduhr and Stenström, Massman and Opitz, Ruskin and Decherd, and other authors.

For calculation of the normal electrical ventricular systole in relation to the heart rate, the following formula should be used (Lepeschkin):

$$Q - T = 2{,}8 \sqrt{R - R}$$

SUMMARY

Two hundred twenty-two electrocardiograms were recorded in 36 rabbits during a period of 20 months. Three standard and four chest leads were used.

Needle electrodes were introduced subcutaneously into the right, left anterior and left posterior extremity; the chest electrodes were introduced at the following points: points I (CR) and IV (CR_5) at the intersection of the vertical line (corresponding to the anterior axillary line) with the horizontal line at the level of the heart; points II and III (CR_1 and CR_4) at the crossing of the parasternal lines with the horizontal. Electrocardiograms recorded with the animal on its back did not substantially differ from those recorded with the animal in the normal position. Five different positions of cardiac electrical axis were observed: normal, left, right, vertical and mixed. These may be spontaneously converted into borderline types. The number of cardiac contractions ranged from 180 to 330 per minute, averaging 250. The duration of the waves and intervals was between 0.01 and 0.02 seconds, depending on the number of cardiac contractions. The value of electric systole depended on the frequency of cardiac contractions. The ECG of the rabbit possesses specific features which should be taken into account in evaluating the cardiac function.

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OSCILLOGRAPHIC RECORDING OF THE BLOOD PRESSURE IN ANIMALS DURING ACUTE EXPERIMENTS

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
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For the oscillographic recording of the blood pressure during acute experiments on animals we have developed a special pick-up, in the construction of which a bellows manometer reading up to 300 mm Hg was used. The pointer and scale were removed, and for them was substituted a steel plate to which were fixed two wire pick-ups with a resistance of 100 ohms. One end of the plate was fixed to the manometer case and the other to the diaphragm of the bellows (Fig. 1). The two tensiometric pick-ups were included in the bridge circuit of a type 8 ANCh-7M tensiometric amplifier.

The output current from the amplifier was transmitted to the loop of the oscillograph. The space inside the bellows was filled with a 4% solution of sodium citrate and connected to one of the animal's arteries. The change in the pressure in the arterial system was transmitted through the diaphragm of the bellows to the plate with the tensiometric pick-ups, thereby leading to a change in the resistance and a disturbance of the balance of the amplifier bridge. The output signal thus produced was proportional to the arterial pressure level (Fig. 2). A different calibration chart is necessary depending on the degree of amplification and on the resistance of the tensiometric pick-ups. Each pick-up that is made thus requires its own calibration data.

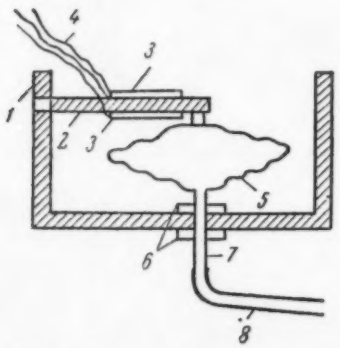


Fig. 1. Diagram of manometer with tensiometric pick-ups. 1) manometer case; 2) steel plate; 3) tensiometric pick-ups, fixed to the plate with BF glue; 4) leads to amplifier; 5) bellows; 6) nut securing bellows to manometer case; 7) metal tube; 8) rubber tube, leading to the animal's blood vessel.

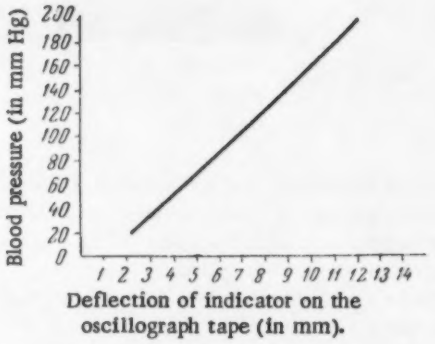


Fig. 2. Calibration chart of the manometer with the tensiometric pick-ups.

The tracings obtained on the oscillograph tape (Fig. 3a) give precise values of the maximum and minimum levels of the arterial pressure at any given moment. It is necessary only to measure the distance between the zero line and the curve of the arterial pressure, when the true value of the latter can be obtained from the graph.

Investigation showed the tensiometric pick-up to be a highly sensitive piece of apparatus for recording the arterial pressure. It has considerable advantages over the mercury manometer, for it completely eliminates the moment of inertia possessed by the mercury. Attention must also be directed to the fact that, when the arterial pressure is recorded at the same time as the electrocardiogram, it is possible to judge the relationship between the time of appearance of the electrical phenomena in the heart (the contraction of the cardiac muscle) and the appearance of the pulse wave in any particular vessel. It will be seen from the oscillogram (see Fig. 3) that the ventricular complex of the electrocardiogram precedes the systolic rise of pressure in the vessel.

The pick-up may be used with success for recording the pressure in the chambers of the heart. For this purpose

a

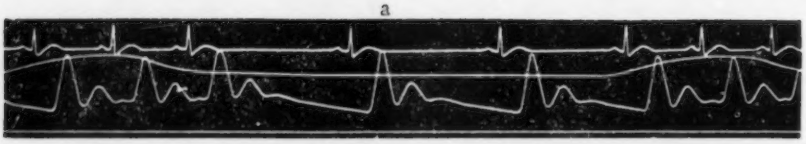
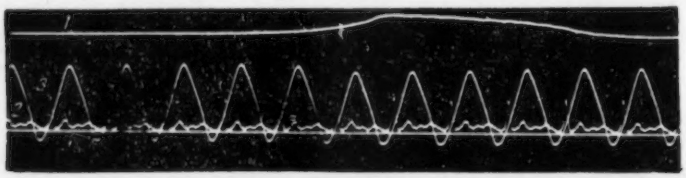


Fig. 3. Oscillogram of the blood pressure in the femoral artery of the dog (a). 1) zero line; 2) blood pressure; 3) respiration, recorded by means of a carbon pick-up; 4) electrocardiogram.

b



Oscillogram of the pressure in the left ventricle of the dog (b). 1) respiration; 2) electrocardiogram; 3) pressure in the left ventricle; 4) zero line.

the bellows of the manometer is connected, by means of a rubber tube introduced through a vessel, with one of the chambers of the heart. The oscillogram (Fig. 3b) shows the curve of the pressure inside the left ventricle of the dog. It may be seen that the pressure curve in the left ventricle (see Fig. 3) has the form of a sinusoid, fluctuating about the zero line, in which the peaks correspond to the value of the pressure arising at the moment of systole.

SUMMARY

A special pressure gauge with tensiometric pick-up was developed to register the blood pressure on the oscillographic tape in acute experiments on animals. The tensiometric pick-ups are included in the resistance bridge circuit of a tensiometric amplifier 8 ANCH-7M, from the outlet of which the signal is transmitted to the oscillograph. The above pick-up may be also used to register the pressure in the chambers of the heart.

METHODS OF ESTIMATING THE DEGREE OF EXPERIMENTAL ATHEROSCLEROSIS

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Translated from Byulleten, Eksperimental'noi Biologii i Meditsiny, Vol. 49, No. 6, pp. 111-113, June, 1960
Original article submitted March 18, 1959

The estimation of the degree of development of experimental atherosclerosis has until recently been highly unsatisfactory. The use of descriptive words (slight, moderate, severe) or the designation of the severity of the process by means of a number of plus signs are totally inadequate for research workers. A great advance was made by K. G. Volkova's suggestion [1] that the degree of atherosclerosis should be judged by the amount of lipids infiltrating the aorta. This biochemical method, notwithstanding its accuracy, has the drawback that it does not permit the area of the lesion to be judged, and it gives no idea of the character of the atherosclerotic process. It becomes necessary to supplement this method by a planimetric device, permitting the distribution of the process to be expressed as a percentage of the total surface area of the aorta. Several authors have suggested that the "atherosclerotic profile" of the aorta be deduced in relation to human postmortem material [2].

The methods which we suggest are as follows.

Gravimetric planimetric method. The aorta, extracted in toto from the semilunar valves to the bifurcation is stretched on glass and, after passing through formalin, is stained in toto with Sudan (Fig. 1a).

The stained aorta is stretched on glass, placed on a board and covered with transparent x-ray film, washed free from emulsion, and is fixed to the board with drawing pins. The contours of the aorta are traced in ink with a fine pen, after which the contours of all the atheromatous elements of the aorta are superimposed. It is especially complicated to trace the area of lesions represented by multiple small plaques and lipoid patches. In these cases it is necessary, to preserve some degree of accuracy, to "unite" the small elements into larger ones, and to outline the postulated contour of the "united" plaques (Fig. 1b).

When the contours of the atherosclerotic elements have been transferred to the film, it is essential to insert a conventional sign inside the contour immediately (as has been done in Fig. 1b), so that during the subsequent treatment of the film the contours of the elements outlined on it may be recognized.

After the aortogram has been obtained, i.e. the contours of the atheromatous elements have been transferred

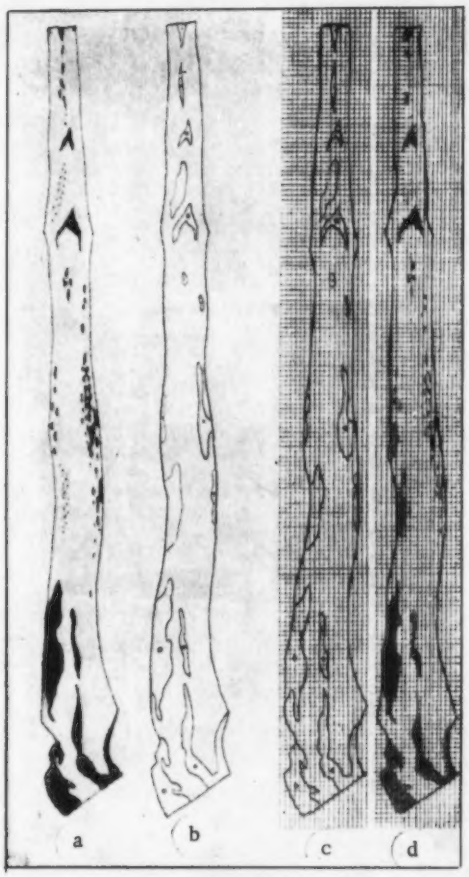

Fig. 1. Methods of planimetric investigation of the rabbit's aorta affected by atheroma. a) aorta of rabbit with cholesterol atherosclerosis (stained with Sudan III); b) aortogram of the same aorta. The large dots inside the contours correspond to large atheromatous plaques, the small dots to thin atheromatous plaques, and absence of sign inside the contour corresponds to lipoid stains; c) aortogram traced on squared paper; d) planimetric grid placed on an aorta stains; with Sudan III.

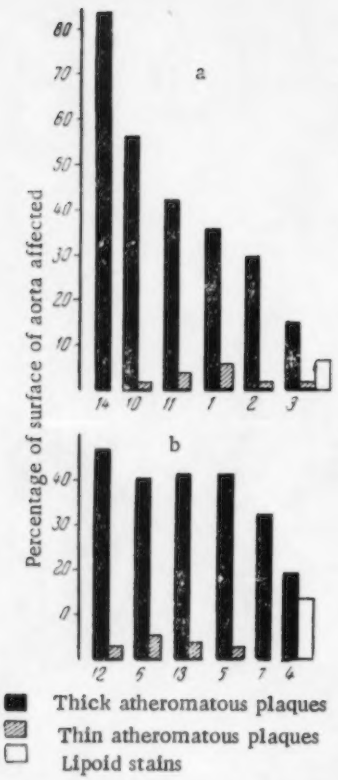

Fig. 2. Graphic illustration of the atherosclerotic formula of the aorta in rabbits. a, b) two groups of animals with comparable degrees of development of atheroma. The figures below the columns designate the number of the experimental animals.

to the film, a piece of the film, which has been removed from the aorta, is cut out corresponding in area to the whole aorta, and weighed on torsion scales. Next, pieces of film corresponding to areas of thick plaques, thin plaques and lipoid stains are cut out in succession.

After the weighing, a simple calculation is made and the atherosclerotic formula of the aorta is deduced. Let us assume that the piece of film corresponding to the surface of the whole aorta weighed 500 mg, and that the pieces of film denoting the area of the thick plaques, the thin plaques and the lipoid stains weighed 50, 100 and 25 mg respectively. Since 500 mg is equivalent to 100% of the surface of the aorta, the atheromatous elements are respectively equivalent to 10, 25 and 5% of the total surface area of the aorta.

For the sake of clarity it is best to give the "atherosclerotic formula of the aorta" in the form of a fraction, in which the numerator is the total percentage of the area of the aorta occupied by atherosclerotic elements, and the denominator gives successively the percentage of the area of the aorta occupied by thick plaques, by thin plaques and by lipoid stains. The atherosclerotic formula of the aorta for the case chosen for the illustration would be of the form: $\frac{40}{10-25-5}$. Where any of the atherosclerotic elements were absent, their place would be occupied by zero or a minus sign.

For the sake of clarity and to enable comparison of the degree of atherosclerosis in groups of experimental animals to be made, the values of the "atherosclerotic formula of the aorta" obtained may be shown graphically (Fig. 2) and in the form of a table.

Method of indirect planimetry. As in the gravimetric method, an aortogram is prepared on x-ray film. This is placed on squared paper (Fib. 1c), after which the number of square millimeters occupied by the whole aorta, and then the number of these on the various atheromatous elements, are counted.

Method of direct planimetry. This method is more precise and more complicated than the preceding method. A planimetric grid is placed beforehand on the transparent film, and through it is counted the number of square millimeters occupied by atheromatous elements, without any transfer of their contours to the film (Fig. 1d).

The use of a planimeter does not give a more accurate estimation of the degree of atherosclerosis than by means of the planimetric grid, because in this case too, the difficulty remains of determining separately the area of each of the smaller atheromatous plaques, on account of the inertia of the apparatus.

Experience of the use of all these methods in practice, in several tens of experiments, enables us to recommend them, although none of them is absolutely accurate. The gravimetric method is less accurate but quicker; the method of direct planimetry is more accurate and less rapid.

SUMMARY

Several planimetric methods are presented by means of which the degree of atherosclerosis may be represented by atherosclerotic formulae. This facilitates the objective evaluation of experimental atherosclerosis.

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THE FORMATION OF DOUBLE AND TRIPLE β-ALANINE STAINS DURING PAPER CHROMATOGRAPHY

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A series of recent investigations has shown that multiple stains may be formed by certain compounds during paper chromatography. These investigations show that great care is necessary when the method of chromatography is used for the identification of substances in biological materials of complex composition.

Multiple stain formation, which has been described for lysine, tryptophane, kynurenin and phenylalanine [1, 2, 4, 6], salicylic and sulfosalicylic acids [5], certain glucosides and antibiotics [3] and sulfuric acid, has not yet been explained. Various workers have put forward the hypothesis that these compounds may form complexes with the solvents, or that a hydrate membrane is formed around the ions, altering their mobility; no convincing experimental proof of these hypotheses has, however, been forthcoming. The most obvious case is that of duplication during chromatography of the optical isomers of certain amino acids, each possessing a different Rf.

METHOD AND RESULTS

We demonstrated duplication of β-alanine stains during chromatography by means of the use of a preparation of this compound in which the carbon of the carboxyl group was labeled with C^{14}.

Trichloroacetic extracts of minced tissues (muscles, liver and kidneys), of which one was incubated at 37° with β-alanine-C^{14} and the others were controls, were freed from trichloroacetic acid by repeated extraction with ether until a weakly acid reaction was given with congo red. The solutions were then evaporated to dryness on a water bath in porcelain dishes and the residue was dissolved in 0.5-1.0 ml of water. Chromatography was carried out in a mixture of butanol, acetic acid and water (5:1:1). After they had been dried, the amino acids gave the usual reaction with ninhydrin (0.2% solution in acetone) on heating. A parallel, undeveloped chromatogram strip was cut into sections, each of which was examined with an end-type counter. The distribution of radioactivity and of the colored stains was compared.

A characteristic picture is shown in Fig. 1. It is seen quite clearly that two or three stains have developed, sharply demarcated from each other, and radioactive. Radioactivity is distributed approximately uniformly between the two large stains; the third stain usually is smaller and less active. The same distribution pattern of stains was shown by both the incubated samples and the controls; the second stain could not, therefore, be a metabolic product of β-alanine. Subsequent experiments showed that two or three stains are also formed during chromatography of control solutions of β-alanine – C^{14} in 2.5% trichloroacetic acid and in water, but only when large amounts (over 40 μg) of β-alanine were used. When smaller amounts of β-alanine – C^{14} (5-20 μg) were applied to the paper, chromatography of pure solutions of the amino acid gave one stain at the level of the standard stain of unlabeled β-alanine. It must be pointed out that the preparation of β-alanine – C^{14} which we synthesized was in the form of well-shaped crystals, with a melting point of 197-198°.

The relationship between duplication of the amino acid stains and concentration is shown in Fig. 2. In this case chromatography was carried out on a solution of β-alanine in a phosphate-saline mixture. Two stains were formed, differing slightly from the usual shape.

Since β-alanine has no optical isomers, it might at first be thought that the additional stains are the result of contaminants, found only with high concentrations of the amino acid. However, the substance responsible for the additional stain was not, in any case, a decarboxylation product of β-alanine (the radioactivity of the carboxyl group was preserved), or a deamination product (staining with ninhydrin). Furthermore, the chemical properties of the β-alanine – C^{14} preparation described above gave no grounds for concluding that any contaminants were present. It was further shown that the "principal" (at the level of the standard solution in a low concentration) and the large "additional" stain could be reduced to a single stain by a change in the reaction of the solution applied to the chromatogram.

In Fig. 3 is shown the chromatogram of β-alanine – C^{14} solutions of different pH. It can clearly be seen that, as the pH increases, the size of the "additional" stain decreases, and finally it disappears altogether, while at the

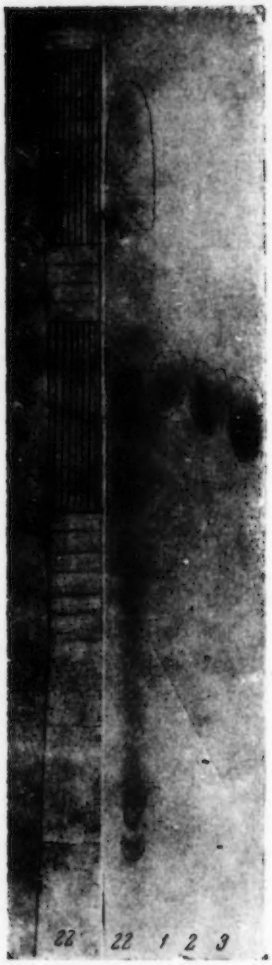

Fig. 1. Chromatogram of a trichloroacetic extract of rat muscle tissue containing β-alanine−C^{14}. 22) trichloroacetic extract + β-alanine−C^{14}; 1,2,3) standard solutions of β-alanine−C^{14}; on the left) chromatogram strip, corresponding to 22, examined with the counter.

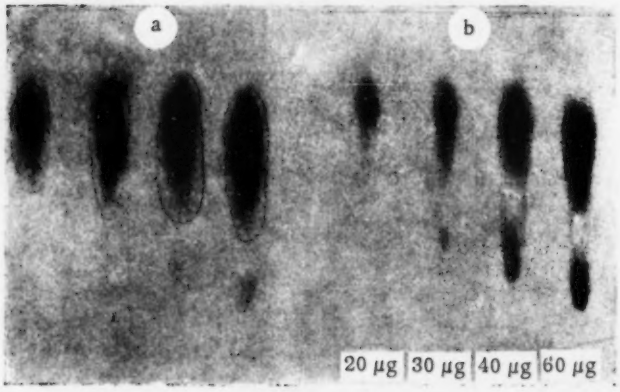

Fig. 2. Chromatogram (a) and the corresponding autoradiogram (b) on x-ray film (exposure 20 days). 20 μg, 30 μg, 40 μg, 60 μg) amounts of β-alanine−C^{14} applied to the chromatogram.

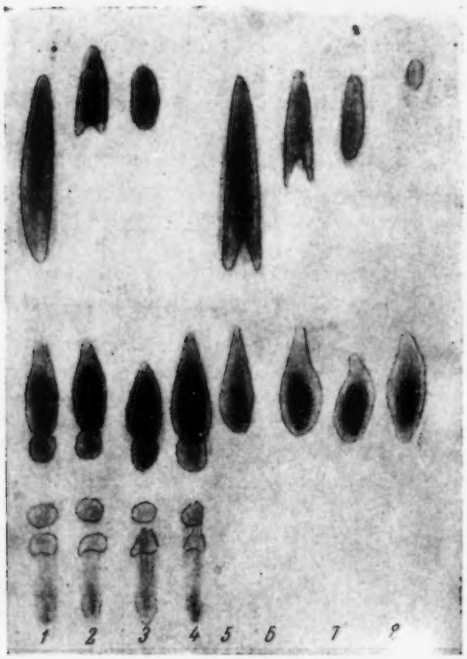

Fig. 3. Relationship between duplication of stains of β-alanine−C^{14} and pH of the amino acid solution applied. 1-4) trichloroacetic filtrate of tissue + β-alanine−C^{14}; 5-8) trichloroacetic acid + β-alanine−C^{14}; 1,5) pH≈3; 2, 6) pH≈6; 3, 7) pH ≈7; 4, 8) pH≈11.

same time the intensity of the "principal" stain increases sharply. The change in pH was brought about by the addition of a solution of NaOH to the weakly acid β-alanine–C^{14} solution.

We cannot yet give an explanation of the duplication of the β-alanine stain which we observed. We must point out, however, that the results of our research, in conjunction with reports in the literature, suggest that uncontrollable conditions may often arise in the process of chromatography, which may distort the results of the determination of the qualitative composition of the mixture undergoing analysis or of the quantitative content of its individual ingredients.

SUMMARY

The formation of double and triple β-alanine–C^{14} spots is seen during chromatography in butanol-acetic mixture; a relationship between this phenomenon and the pH of the β-alanine solution applied on the paper is also apparent. The author discusses the importance of this phenomenon for evaluation of the results of qualitative and quantitative investigations of the composition of complex biological mixtures.

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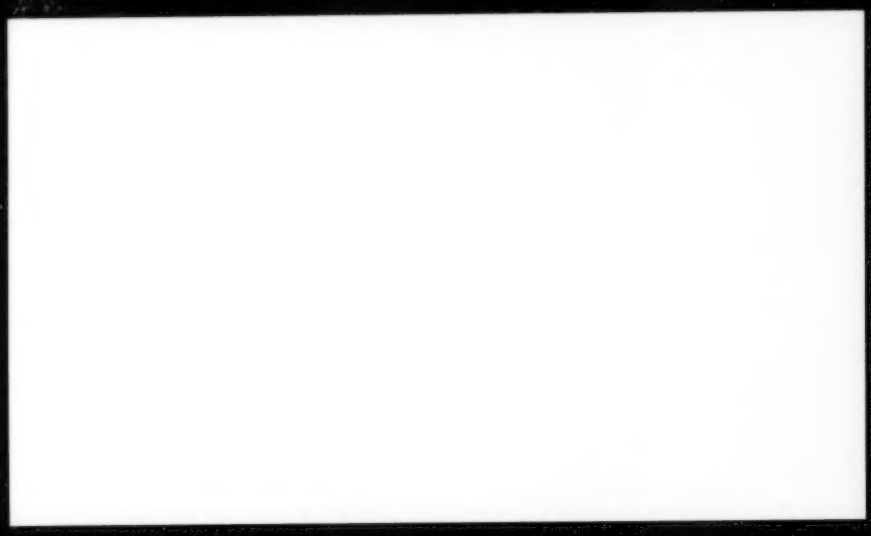

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspt. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PROCEEDINGS OF THE ALL-UNION SCIENTIFIC AND TECHNICAL CONFERENCE ON THE APPLICATION OF RADIOACTIVE ISOTOPES MOSCOW, 1957

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